ANTIHYPERLIPIDAEMIC AND ANTIOXIDANT ACTIVITY OF *ARTOCARPUS HETEROPHYLLUS* STEM EXTRACT

A Dissertation submitted to

The Tamil Nadu Dr.M.G.R Medical University Chennai

In partial fulfilment of the degree of

MASTER OF PHARMACY

(Pharmacology)

Submitted by

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CERTIFICATES

CERTIFICATE

This is to certify that this dissertation entitled ***"Antihyperlipidaemic And Antioxidant Activity Of Artocarpus Heterophyllus Stem Extract"*** Submitted by **Mr. Ubaid.K** to The Tamil Nadu Dr.M.G.R Medical University, Chennai in partial fulfilment for the degree of **Master Of Pharmacy In Pharmacology** is a bonafied work carried out by the candidate under my guidance and supervision in the Department of Pharmacology, Karpagam College of Pharmacy Coimbatore-32.

I have fully satisfied with his performance and work. I have forward this dissertation work for evaluation.

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DECLARATION

I hereby declare that this dissertation entitled **"*Antihyperlipidaemic And Antioxidant Activity Of Artocarpus Heterophyllus Stem Extract*"** submitted by me, in partial fulfilment of the requirements for the degree of **Master Of Pharmacy In Pharmacology** to The Tamil Nadu Dr.M.G.R Medical university, Chennai is the result of my original and independent research work carried out under the guidance of **Mr.G. Nagaraja Perumal,M.Pharm.,(Ph.D)** Professor & Head Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore-32, & Co-Guide **Dr. R.S. Parihar, Banner Pharmacaps,** during the academic year 2016- 2017.

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EVALUATION CERTIFICATE

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Date:

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DEDICATED TO MY BELOVED

PARENTS, TEACHERS AND ALMIGHTY

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CHAPTER I

1.INTRODUCTION

1.1 Hyperlipidemia

Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations. Hyperlipidemia may be caused by specific genetic abnormalities called primary hyperlipidemia6 or may be idiopathic caused by lifestyle habits or medical diseases such as diabetes, kidney disease, pregnancy, hypothyroidism and heart disease.

Hyperlipidemia prevalence continued to increase annually, requiring the develop-ment of drugs capable of lowering blood lipids to reduce mortality and morbidity due to cardiovascular complications. Although synthetic lipid-lowering drugs are useful in treating hyperlipidemia, there are number of adverse effects. So, the cur-rent interest has stimulated the search for new lipid-lowering agents with minimal side effects from natural sources.

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history. In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects when comparing other system of medicine. India being the botanical garden of the world with more than 2400 medicinal plants out of 21000 species being listed by WHO, is the largest producer of medicinal plants around the globe.

*Artocarpus heterophyllus*is a large, evergreen tree, 10-15m in height, indigenous to the evergreen forests at altitude of 450-1,200m and cultivated throughout the hotter parts of India. Stem of this plant is straight rough whereas bark is green or black, 1.25cm thick, exuding milky latex, leaves

broad obovate, elliptic, decurrent, glabrous, entire inflorescence solitary axillaries, cauliforous and ramflours on short leafy shoots. Male head is sessile or on short peduncles receptacles, sometimes born on the ultimate twing, Female head are oblong ovoid receptacle, syncarpus, cylindrics. Seeds are separated horny endocarpus enclosed by sub-gelatinous exocarpus (1mm thick) oblong ellipsoid in nature. The sweet yellow sheaths around the seeds are about 3-5 mm thick and have a taste similar to that of pineapple, but milder and less juicy. Even though it is well known for its antibacterial, anti-inflammatory, anti-diabetic, antioxidant and immunomodulatory properties there are no evidences regarding the anti-hyperlipidaemic effect of the stem hence our study has its relevance

The biggest organ in the body is the "LIVER" and it is likewise fills in as the essential metabolic organ of the body. In spite of the fact that the liver is comprised of various cells like hepatocytes, endothelial, kupffer and stellate cells are the most dominating with critical capacities. Another most essential one of a kind component of the liver is its capacity to recover. Well grown-up liver (i.e. Grown-up) is the standard organ accountable for detoxifying and metabolizing, exogeneous/endogenous mixes, rendering them more hydrophilic, which as often as possible impact their force and action1.

Liver infections are the genuine restorative issues went up against by the people wherever all through the world. The epidemiological review demonstrates that around 20,000 passings happen reliably in light of liver issue. In Africa and Asia, the major driver of liver maladies are contaminations by infection and parasite, while in Europe and in North America, a vital reason is liquor manhandle. Liver ailments are primarily realized by deadly chemicals, over the top affirmation of ceaseless liquor, diseases and immune system issue. Hepatic harm by over measurements of drug appears, from every angle, to be a run of the mill contributing component. Liver is required to do physiological limits and additionally to guarantee against the perilous of dangerous drugs and chemicals. Prescription impelled substance damage is accountable for 5% of each mending focus attestation and half of all serious

liver disappointment. Over 75% of episodes of specific prescription reactions achieve liver transplantation or death2.

1.2 Pathophysiological Mechanisms

Pathophysiological components of hepatotoxicity are as yet being found and contain both hepatocellular/extracellular systems.

Disturbance of hepatocyte: Medications can bound to intracellular proteins by covalent tying which realize a reducing in ATP levels inciting actin intrusion. Some portion of actin fibrils at the surface of the hepatocyte causes blebs and burst of the layer.

1.3 Interruption of transport protein

Bile stream might be hindered by meds that impact transport proteins at canalicular film. Loss of villous strategies and interruption of transport pumps, for instance, multidrug resistance-related protein 3 hinder release of bilirubin realizing cholestasis

Cytolytic T-cell actuation: Co-valent binds of pharmaceutical to Cytochrom P-450 compound goes about as an immunogen enacting T-cells and cytokines and energizing multifaceted safe responses.

Apoptosis of hepatocytes: Enhancement of apoptotic pathways by tumor rot calculate alpha receptor of Fas may trigger the course of intercellular caspases, which achieve altered cell passing.

Mitochondrial disturbance: A couple of meds limit mitochondrial limit by twofold effect on both beta-oxidation vitality creations by frustrating the union of nicotinamide adenine dinucleotide and flavin adenine dinucleotide, realizing decreased ATP era.

Bile pipe damage: Dangerous metabolites discarded in bile may achieve mischief to bile course epithelium3.

Solution/sedate incited liver harm is a prosperity issue, and is depended upon to increase as the amount of drugs being eaten up augmentations, both remedy and non-solution, and in view of the present example of usage of

pharmacologically dynamic substances in correlative and option prescription. Prescription/tranquilize incited hepatotoxicity is the most surely understood reason alluded for withdrawal of authoritatively endorsed meds from the business. It also speaks to more than 50 percent of occasions of serious liver disappointment in the United States. The positive recurrence of solution/medication incited liver harm is difficult to gage, and all things considered, concentrates going to measuring its event encounter the evil impacts of drawbacks, for instance, under-detailing and that data by expansive start from audit thinks about. Frequently, there is in like manner a nonattendance of information about self-arrangement and use of home grown item that may associate with prescription and non-doctor embraced medications4.

Despite the repeat of solution actuated liver harm being low, data from the Centers for Disease Control and Prevention in the U.S. report pretty much 1600 new extraordinary examples of liver disappointment yearly, of which Paracetamol hepatotoxicity speaks to plus or minus 41%. Exactly when taking a sexual orientation at hospitalized patients, the rate of hostile solution reactions is assessed to be 6.7%, and deadly disagreeable medicine reactions mean 0.32%, as controlled by a meta-investigation of around 40 imminent reviews. Amid the period 1995 to 2005, the reports of horrible prescription reactions and also passings related to these, have drastically increased. Various examples of pharmaceutical activated liver harm are particular, i.e. the reaction is whimsical considering the known pharmacological properties of the medication, and from this time forward is scarcely perceptible amid preclinical periods of change. There are however studies to demonstrate that these reactions might be liable to an extended affectability of the patient to the medicine being alluded to, dependent upon such segments as other going with contaminations or other relating prescriptions. Certain innate factors, for instance, HLA-sort, can once in a while add to the affectability of a man to opposing pharmaceutical reactions. Commonly, clinically clear hostile drug reactions happen when some season of inertness, wherever in the compass going from one to 12 months (most by and large within 90 days), and about constantly vanish after departure of the

solution. Pharmaceutical researched liver damage may give a couple of unmistakable clinical segments; hepatitic/hepatocellular, cholestatic or mixed.

Regardless of their etiology, solution/tranquilize prompted hepatotoxicity remains a significant issue amid medicine advancement in the pharmaceutical business, both concerning extended threat for patients encountering clinical trials, besides tolerant hazard after the acquaintance of new drug with the treatment. Moreover, because of the extended costs that take after disappointment of a prescription to-be at a late stage in medicine improvement or after its launch6.

1.4 Drug Toxicity Mechanisms

Commonplace division of pharmaceutical responses is of at the very least 2 significant events which include:

- Drugs which clearly impact liver.
- Drugs which intervene a safe response.

Characteristic/unsurprising medication responses: Drugs that has a place into this characterization cause reproducible injuries in creatures and mischief is related to measurements. Mischief can be a result of medicine itself or to its metabolite. Acetaminophen is the most proper delineation of a known regular or obvious hepatotoxin at supertherapeutic measurements. Another outline is carbon tetrachloride.

Quirky/eccentric medication responses: These medication reactions can be portioned into those that are named excessive touchiness or immunoallergic and those that are metabolic-particular. It occurs without evident measurement reliance and in an unusual manner

Hepatotoxicity to a great extent demonstrates the compound constrained liver devastation. A few drugs when devoured in overdose and once in a while notwithstanding when taken inside suggest measurement may harm numerous inner organs. Few compound/substances involving those that are utilized as a part of research facilities (Example: CCl4 and Paracetamol) and ventures (Lead, and arsenic) and characteristic mixes (microcystine and aflatoxins) and home grown treatments (cascara sagrada, ephedra) can

likewise root hepatotoxicity. Chemicals/Compounds that cause liver harm are as one marked as hepatotoxins.

NSAIDS (Acetaminophen, Aspirin, Ibuprofen)

Glucocorticoids.

Against Tubercular medication (Isoniazid).

Mechanical poisons (arsenic, carbon tetrachloride, vinyl chloride).

Natural cures (Ackee organic product, camphor, cycasin, kava leaves, valerian, comfrey).

1.5 Alcohol liquar Hepatotoxicity

Liquor is one of the key inducer of end-stage liver harm far and wide. In the United States, alcoholic liver malady is the second most normal reason behind liver transplantation. The Dionysos Study, an accomplice examination of the transcendence of unending liver malady in an Italian people, showed that 21% of the masses considered was at peril for making liver harm. Of these, only 5.5% of the general population at peril implied at genuine liver harm. Around 50 years earlier it was acknowledged that liquor in itself was not destructive, rather that the dietary deficiencies every now and again running as an inseparable unit with it were the genuine purposes behind liver damage. Regardless, it was demonstrated by Lieber and De Carli that in rats, alcoholic liver harm made despite satisfactory sustenance. The lethality of liquor was later on exhibited to be related to its absorption framework by liquor dehydrogenases (ADHs) moreover to the assimilation framework by CYP2E1. There is also a piece of assimilation framework by catalase. The basic pathway for ethanol EtOH oxidation in the liver is by method for ADH to acetaldehyde, which is associated with the diminishment of NAD to NADH. NADH in this way constructs xanthine oxidase activity, which rises era of superoxide. Metabolic arrangement of EtOH by liquor dehydrogenase impacts the redox status of the liver in like manner in various ways. Lifted acetaldehyde creation after EtOH digestion system decreases hepatic glutathione (GSH) content. The reducing in GSH is both on account of an extended disaster, and furthermore a lower rate of blend

Ethanol prompts number of harmful metabolic changes in liver. Admission of ethanol for long time prompts to advancement of steatosis, alcoholic hepatitis and cirrhosis bringing about weight and volume changes. Around 80% of overwhelming consumers had been accounted for to create steatosis, 10-35% alcoholic hepatitis and roughly 10% liver cirrhosis.

1.6 System hidden Ethanol actuated hepatotoxicity

Liquor utilization brings about increment in arrival of endotoxin from gut microbes and layer porousness of gut to endotoxin or both. Females are all the more frequently touchy to these progressions. Blood endotoxin is lifted and enters liver where it is overwhelmed by Kupffer cells that get to be distinctly actuated discharging TNF-alpha, PGE2 and free radical. Prostaglandins increment oxygen take-up and are in charge of hypermetabolic state in liver. Increment in oxygen request prompts to hypoxia of liver and on reperfusion alpha - hydroxyethyl free radicals are framed that prompts to tissue harm in oxygen poor pericentral districts of liver lobule. Obstructing of these occasions should be possible by sanitization of gut utilizing anti-infection agents or decimation of Kupffer cells with Gdcl3 and in this manner averts liver injury8..

1.7 Symptoms of Hepatotoxicity

signs and side effects delineated in different foundations for Hepatotoxicity incorporate 15 side effects as recorded below:

- Sickness
- Stomach torment
- Loss of hunger
- Looseness of the bowels
- Tiredness
- Shortcoming
- Jaundice
- Yellow eyes
- Regurgitating
- Yellow skin
- Hepatomegally
- Irregular liver capacity test comes about
- Swelling in feet
- Weight increase because of water maintenance
- Delayed draining time.

1.8 Treatment for Hepatotoxicity

The rundown of medications specified in different hotspots for hepatotoxicity incorporates the accompanying. Continuously take after expert restorative guidance about any treatment or change in treatment arranges. Treatment of hepatotoxicity is relies on causative operator, level of liver brokenness and age and general strength of patient. Medicines for hepatotoxicity include:

Withdrawal of causative solution or expulsion from introduction to causative specialist.

General checking of patient and survey of liver capacity – where liver brokenness is mellow to direct and liver capacity is moving forward.

Finish shirking of liquor and drug that may add to further liver harm.

N-Acetylcysteine is utilized for paracetamol harmfulness.

Administration of indications of liver harm.

- Nutrition – with vitamin supplementation as required
- Regular practice with a specific end goal to keep up bulk.
- Ursodeoxycholic corrosive.

Administration of pruritus

- Cholestyramine
- Antihistamines.

Administration of ascites

- Low sodium eat less carbs.
- Diuretics – furosemide, spironolactone.
- Removal of liquid through a needle in the stomach area – Paracentesis.
- Portosystemic shunting.

Administration of entry hypertension

- Beta – blockers
- Oesophageal variceal banding

- Portocaval shunt
- Administration of intense liver disappointment because of hepatotoxicity
- Supportive care dependably in emergency unit aviation route assurance, liquid and electrolyte administration.
- Management of intricacies, for example, draining issues and hepatic encephalopathy.
- Liver transplantation – for intense fulminant liver disappointment or end organize cirrhosis.

1.9 Present day Medicines for Treatment of Liver Diseases

Liver illnesses can be dealt with utilizing allopathic and in addition by utilizing home grown medications.

1.10 Hepatoprotective Allopathic Treatment

Couple of present day drugs are accessible for treating liver illnesses that incorporates:

Ursodeoxycholic corrosive (Ursodiol): Ursodiol diminishes intestinal retention and stifles hepatic union and capacity of cholesterol. It is predominantly utilized as a part of administration of constant hepatic ailments in people.

Penicillamine: Penicillamine chelates a few metals like copper, iron, lead and mercury shaping stable water dissolvable edifices which are renally discharged.

1.11 Different medications: Antiviral pharmaceutical, for example, alpha interferon, ribavirin, steroids, anti-infection agents and so on are additionally utilized as a part of liver ailments. Drugs like tricholinecitrate, trithioparamethoxy phenyl propane, basic phospholipids, blend of medications, for example, L-ornithine, L-aspartate and pancreatin, silymarin and Ursodeoxycholic corrosive are generally recommended for hepatitis, cirrhosis and other liver sicknesses. N-acetylcysteine is utilized as a part of early periods of acetaminophen harmfulness. L-carnitine is conceivably

significant amid valproate poisonous quality. Cholestyramine can be utilized to mitigate pruritus.

1.12 Inconveniences of allopathic medications

Symptoms of numerous cutting edge medications are generally disturbing. Collaborations, contra-cooperations, reactions and danger of engineered pharmaceutical shift from gentle to extreme that incorporates sleep deprivation, regurgitating, weariness, dry mouth, looseness of the bowels, blockage, tipsiness, self-destructive thought, despondency, seizures, pallor, male pattern baldness, high glucose, swelling, impotency, perplexity, blacking out lastly passing. Anti-microbials more often than not bring about stomach furious or unfavorably susceptible responses. Interferon indicates symptoms as influenza like ailment with fever and body throbs.

1.13 Natural Hepatoprotective Drug Treatment

Various polyherbal arrangements have been utilized as a part of treating different liver issue since ages. Some natural definitions include:

1.14 Constraints of natural arrangements

Natural based arrangements for treating liver issue has been utilized as a part of India for long time and has been promoted worldwide by selling pharmaceuticals. Regardless of prevalence of home grown prescriptions for liver illnesses specifically, are still inadmissible treatment modalities for liver sicknesses. Constraining variables include:

- Lack of institutionalization systems of home grown arrangements.
- Lack of recognizable proof of dynamic parts and standards.
- Lack of randomized controlled clinical trials (RCTs).
- Lack of toxicological evaluation67.
- Poor solvency.
- Poor bioavailability.
- Poor hepatic cell recovery.

1.15 Hepatoprotective Mono-Herbal Medicines

Restorative plants are critical wellsprings of hepatoprotective medications. Very nearly 160 phytoconstituents from 101plants have been guaranteed by Pharmacopeia Foundation to have hepatoprotective action11. Home grown medications are most generally utilized than allopathic medications as hepatoprotectives in light of the fact that these are normally cheap, better social adequacy, enhanced similarity with human body and insignificant symptoms. Different classes of phytoconstituents like flavonoids, triterpenes, lignans, steroids, glycosides, polyphenols, saponins, coumarins and unpredictable oils and so forth gangs hepatoprotective action.

Basically diabetes is characterized by hyperglycemia, a condition of lack of insulin and development of complications in nephrons of kidney, peripheral nerves and retinal damages. Considerable effect on heart has also be the problem of developing further complications leading to atherosclerotic threats to brain, myocardium and lower extremities. Hyperglycemia causes various kind of injury to vascular system viz, increased pace of high glucose flux, intracellular production of advanced glycation products, activation of protein kinase and abnormal hexoseamine pathway. The increased mitochondrial reactive oxygen species (ROS) would lead to microvascular changes heart and other vital functions of organs and their complex pathways . The damage breaks out by ROS production both mitochondrial and non-mitochondrial results in, tumor formation, age-related degeneration, inflammatory conditions and diabetes mellitus [. Better understanding of ROS production and its intervention strategies leading to solution to this problem with newer technologies. In this context major factor for onset of diabetes has been evidenced due to ROS generation . Further various animal studies confirm that embryos are more vulnerable to the oxidative stress especially in type 2 diabetes. Maternal abnormalities were developed and observed to be more prominent in heart and reduction in pregnancy of the animals has been notified .The existing methods of treating diabetes do not combat diabetic complications, so there is an increased need for effective treatment, which is essential to fight with diabetic complications in relation to considerable reduction of ROS by using various technology and herbal drugs.

1.16 Consequences for Insulin Resistance and ROS production

In a condition pertaining to the high plasma levels of glucose and free fatty acids leads to increased production of reactive oxygen species (ROS) and to a least of reactive nitrogen species (RNS). In turn the initiation of various kinases starts occurring; proceed to phosphorylation of the insulin receptor and nitric oxide generation . Both the aforementioned pathways cause the signaling of insulin and suppress it drastically. Cascading reactions lends increased insulin resistance in liver, skeletal muscle and adipose tissues.As shown in the Fig 1.

Increased free fatty acid level and lipid content are the prime factor for insulin resistant type 2 diabetes. Besides, the production of ROS could be more due to free fatty acids are common and mitochondrially how ROS is produced is still not understood and yet to be explored.

1.17 ROS and associated Hypertension

The considerate onset of hypertension is due to the non-phagocytic NAD(P)H oxidase (Nox1, Nox 2 and Nox 4), apart from other factors for increased diabetic and hypertensive complications such as mitochondrial generation, inflammation, hypertrophy apoptosis, fibrosis, angiogenesis and rarefaction. Miscellaneous occurrence for ROS bounds to xanthine oxidase, cyclooxygenase, lipoxygenase and nitric oxide synthase. As shown in the Fig 2.

Normal physiological processes affected by ROS are immunity, endocrine functions, embryogenesis and signal transduction at cellular level [13]. The intervention has given a tool to effectively control the ROS generation by antioxidants or nitric oxide production, to minimize the vascular injury, renal dysfunction and prevent target organ damage in diabetes and hypertension

1.18 Delayed Wound Healing Pattern – Increased ROS

Scoring up of ROS generation would lead to delayed wound healing, as it is the significant clinical problem to deal with to treat with different approach.

Antioxidants have forecasted evidence for healing process to be very effective if it is provided. a study has provided robust substantiation in cultured fibroblast, a diabetic phenotype and IGF1, which promotes wound healing on exposure to antioxidants. Pre-treatment of antioxidants increased the IGF1 has brought down diabetic complication and accelerate wound healing .

1.19 Basis for Diabetic and ROS

Long term diabetic causes are

i. Excess nourishment (Food)

ii. sedentary life style

iii. genetic or miscellaneous factors

All the above conditions leads to glucose and fatty acid overload, in addition the reaction of glucose with plasma proteins forms glycation end products and ROS. The ROS which in turn causes increased non-availability of nitric oxide, increased inflammatory mediators and modification of lipoproteins in atherosclerotic condition.

Common complications of diabetes due to ROS are

i. development of insulin resistance

ii. *β*- cell dysfunction

iii. type 2 diabetes

iv. increased glucose tolerance

1.20 Diabetic Nephropathy and ROS

ROS play an important role in commencement and progression of diabetic nephropathy. The roles of oxidative stress in pathogenesis of diabetes complications are evidenced. Vulnerability to glomeruli and retina is observed

in patients with insulin resistance diabetes. A ROS-regulated signaling pathway leads to extracellular matrix (ECM) deposition in diabetic kidney was evidenced. ROS are increased in the glomeruli isolated from streptozotocin diabetic rats, providing a direct evidence of increased ROS in diabetic glomeruli.

An approach positively controls the nephritic damages are the treatment with antioxidants. As Antioxidants effectively inhibit high glucose and H_2O_2 induced activation in case of diabetic nephropathy, which would favor patients. The effect of antioxidant therapy is well documented in cell and animal studies, although convincing evidence for clinical efficacy is still lacking.

Exhaustive glycemic control and inhibition of angiotensin II delay the onset and progression of diabetic nephropathy, in part, through prevention of overproduction of ROS. Antioxidants have been shown to prevent or delay the onset of diabetic nephropathy and its progression.

1.21 Role of ROS in insulin resistant type 2 diabetes

Receptor level binding of insulin at cell surface leads to the phosphorylation and various signaling pathways, which has been affected by ROS with increased insulin resistance and pancreatic cell dysfunction. Therapy with antioxidants like N-acetyl-L-cystine and taurine prevents the hyperglycemia induced by insulin resistance. In patients with type 2 diabetes, acute and chronic administration of lipoic acid, antioxidant, improved insulin resistance.

1.22 Role of Herbal antioxidants in ROS

The damaging effects of ROS is tackled effectively by antioxidants, normally superoxide and hydrogen peroxide are produced in the body. If excess quantities of generation leads to pathological ROS production. Many herbs has the potential to compromise ROS such as green tea, grape seed, ginseng and Scutellaria baicalensis. Long while herbal medicines used for the diabetes has been in existence. Current pre-clinical and clinical studies have demonstrated that many of them exhibit potent anti- inflammatory and anti-oxidative properties, and have also identified the active phytochemicals

responsible for their activities. The herbal medicines and nutraceuticals, as well as their bioactive components, which exhibit anti-inflammatory and anti-oxidative properties, provide a promising approach for the prevention and treatment of diabetic complications. The etiology of diabetes and its complications are because of free radicals and for the reason herbs with antioxidant properties are believed to possess faith in controlling and minimizing the damage due the reactions. The list of some herbs used for diabetes and its complications are given in Nearly 400 herbs are accounting for diabetes treatment worldwide.

Acacia arabica (Babhul) has got anti-diabetic agent shown to have hypoglycemic effect. Aegle marmelos (Bengal Quince) which improves digestion and reduces blood glucose, urea and serum cholesterol level. Allium cepa (Onion) is a potential antioxidant, anti hyperglycemic and anti hyperlipedemic activity. Allium sativum (Garlic) has been used to increases insulin secretion and controls lipid peroxidation. Aloe vera stimulates β cell to secrete insulin, Anti-inflammatory and wound healing. Azadirachta indica (Neem) evidenced using anti-hyperglycemic, hepatoprotective and antioxidant activities. Eugenia jambolana (Jamun) is a viable anti-hyperglycemic agent. Mangifera indica (Mango) is a anti-diabetic agent, reduces intestinal glucose uptake. Momordica charantia (Bitter gourd) is utilized as antidiabetic and antihyperglycemic Agent. Ocimum Sanctum (Holy basil) cause glucose level decline in fasting condition, triglyceride and total lipid content. Phyllanthus amarus (Bhuiawala) is a antinflammatory, anticancer, antioxidant and antidiarrhoeal. Certain formulations available for the diabetic treatments are given table 2

Bao H et al studied icariin a flavonoid of Epimedium pubescens known to have considerable antioxidant activity . They demonstrated cardiac functions and mitochondrial oxidative stress in streptomycin induced diabetic rats. The observations are in favor of controlling oxidative stress of cardiac complications in diabetes induced animal. An 8 weeks of administration markedly improved cardiac function and ROS has been proved effectively.

The nanotechnology is facing expansions in all dimensions for serving mankind, that almost all the countries are striving to explore for the social well being and economy of the country. Nanoparticles are known to have tremendous applications in the field of diagnosis and theraphy. Such imperative nanoparticles have very great trait to carry and serve like an antioxidant, antihyperglycemic and ROS interfering action. Treatment of antidiabetic potent nanoparticle with plants would have therapeutic value do create a new platform for herbal medicines in nanoscience for drug delivery. Intentions of few antidiabetic nanoparticles of herbal origin are discussed. the options for herbal nanotechnology is shown in the fig 3.

Feng lin et al have demonstrated the preparation of nanosuspension of Cuscuta chinensis, since its principles are majorly flavonoids which has got poor solubility. It drives them to make it more soluble formulation. The prepared formulations are tested with acetaminophen induced hepatotoxic rats. As the flavonoids are known to have antioxidant which has the caliber to control oxidative stress these components (flavonoids) are taken into account in this study. They observed only 50mg/kg of body weight of nanosuspension containing Cuscuta chinensis, effective than 125mg/kg weight administered from ethanolic extract of same drug. In this context suggestions are given to increase the tough molecules solubility enhancement through nanotechnology

1.23 High antioxidant activity of Dalbergia sissoo (Indian Rosewood)

Nayan Roy et al studied extracts of the plant stem bark, they intervened to extend they work towards invitro antioxidant determination by chemical method, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. In their experimentation of aqueous and methanolic extract they found aqueous extact has greater activity. They concluded that plant has high antioxidant activity and it may find it very useful in the treatment of diseases and complications caused by oxidative stress.

1.24 Nanoencapsulation of Albizia chinensis

Avnesh kumar et al explained the nanoencapsulation of the herb having potential antioxidant activity of its content quercitrin. The polymer poly-D,L-

lactide (PLA) is used to encapsulate the material and solvent evaporation technique was deployed to prepare the nanodimensions of the drug. The drug quercitin was made to encapsulate to increase the solubility, permeability and stability of the molecule. Moreover, the properties of nanomedcine has provided a new potential use of less useful highly active antioxidant molecule towards the development of oxidative stress related inflammation and its related complication profiles.

1.25 Antioxidant enriched Siylmarin Nanoparticles

Xia cao et al ventured in developing the porous silica nanoparticles of silymarin to increase the solubility as it has the considerable antioxidant activity. The silymarin nanoparitcles were prepared by porous microemulsion and ultrasonic corrosion methods. The results are bioavailability of silymarin was considerably increased despite the drugs basic poor solubility nature. The evidences are strong that herbal components are appropriate option for oxidative stress management in excessive ROS generation.

1.26 Metal andioxidant nanoparticle

As antioxidants have significant role in influencing ROS, such antioxidant nanoparticles are prepared from metals such as gold, silver and so on. These methods of producing metal antioxidant nanoparticle using plant extracts are extremely biosynthesized. Kannan et al explored syhthesis of gold nanoparticles using leaf extracts of Coleus amboinicus. The prepared nanoparticles are characterized by UV-vis spectroscopy, XRD, TEM and SAED analyses. This method utilizes cheap production of nanoparticles with non toxic nature. Praveenkumar et al studied gold nanoparticle synthesis using Zingiber officinale extract. They got nanoparticles of size range 5 to 15 nm, and Zingiber officinale as stabilizing and reducing agent which is more potent than asprin. Characterization was done by Dynamic Light scattering

(DLS), TEM and UV-Vis Spectroscopy. The produced nanopartcles are biocompatible with the blood has been observed [35].

Diabetic treatment channelizing to the effective control of glucose level and specific strategy to target the ROS generating pathway curbing, do produce better results and compliments each other beneficially. A biological antioxidants capable of restraining oxidative stress mediated diabetic complication in due course of hyperglycemia is still mandatory to foresee better clinical improvements. The antioxidant enriched herbal components is the viable tool to cope with oxidative stress condition in diseased condition especially, the diabetes. Secondly, evidences are there that such components of antioxidant, antidiabetic and hypoglycemic herbs are tailored to nanotization for the maximum benefit. Provided with the strong scientific back up evidences, the clinical implications of nanotechnology based herbal constituents such as antioxidants are in great need to the mankind, to fight with oxidative stress related complications in diabetes and related ailments.

Table No 1: List of some herbs for diabetes and its complications

Botanical name	**Common/Vernacular Name**
Eugenia Jambolana	Indian Gooseberry
Momordica charantia	Bitter gourd
Ocimum sanctum	Holy Basil
Phyllanthus amarus	Bhuiawala
Pterocarpus marsupium	benga
Tinospora cordifolia	Guduchi

Trigonella foenum	Fenu greek
Withania somnifera	Ashwagandha
Allium sativum	Garlic

Table No 2: List of herbs and its intention to intend

Name of Herb	**Common/Vernacular**	**Intention/purpose**
Acacia arabica	Babhul	Anti-diabetic agent shown to have hypoglycemic effect.
Aegle marmelos	Bengal Quince	Improves digestion and reduces blood glucose, urea and serum cholesterol level
Allium cepa	Onion	Antioxidant, anti hyperglycemic and anti hyperlipedemic activity

Allium sativum	Garlic	Increases insulin secretion and controls lipid peroxidation
Aloe vera	Kathalai	Stimulates β cell to secrete insulin, Anti-inflammatory and wound healing
Azadirachta indica	Neem	Anti-hyperglycemic, hepatoprotective and antioxidant activity
Eugenia jambolana	Jamun	Anti-hyperglycemic
Mangifera indica	Mango	Anti-diabetic agent, reduces intestinal glucose uptake.
Momordica charantia	Bitter gourd	Antidiabetic and Antihyperglycemic Agent
Ocimum Sanctum	Holy basil	Glucose level decline in fasting condition, triglyceride and total lipid content
Phyllanthus amarus	Bhuiawala	Antiinflammatory, anticancer,

		antioxidant and antidiarrhoeal

Jackfruit (*Artocarpus heterophyllus*) is one of the most significant trees in tropical homegardens and perhaps the most widespread and useful tree in the important genus *Artocarpus*. It is a medium-size evergreen tree typically reaching 8–25 m (26–82 ft) in height that is easily recognized by its fruit, the largest among cultivated plants. The succulent, aromatic, and flavorful fruit is eaten fresh or preserved in myriad ways. The nutritious seeds are boiled or roasted and eaten like chestnuts, added to flour for baking, or cooked in dishes. It is also known for its remarkable, durable timber, which ages to an orange or red-brown color. The leaves and fruit waste provide valuable fodder for cattle, pigs, and goats. Many parts of the plant including
the bark, roots, leaves, and fruit are attributed with medicinal properties. Wood chips yield a dye used to give the famous orange-red color to the robes of Buddhist priests. The tree can provide many environmental services. It is highly wind tolerant and therefore makes a good component in a windbreak or border planting. Growing in pastures, it can provide fallen fruit for livestock, shade, and long-term timber. In homegardens, the dense jackfruit canopy can provide a visual screen and is very ornamental. Introduced to most Pacific islands after European contact, the tree can be found throughout the Pacific, mainly in homegardens, where it finds a place among other favorite multipurpose plants. It is easy to grow and more adaptable than some of the other common *Artocarpus* species such as breadfruit (*A. altilis*). It is not considered to be an invasive species.

1.27 Distribution

1.27.1 Native range

The tree is reportedly native to the rainforests of Malaysia and the Western Ghats of India.

1.27.2 Current distribution

Jackfruit has been cultivated since prehistoric times and has naturalized in many parts of the tropics, particularly in Southeast Asia, where it is today an

important crop of India, Burma, China, Sri Lanka, Malaysia, Indonesia, Thailand, and the Philippines. It is also grown in parts of

Africa, Brazil, Suriname, the Caribbean, Florida, and Australia. It has been introduced to many Pacific islands since post-European contact and is of particular importance in Fiji, where there is a large population of Indian descent. In a 1985 survey, jackfruit was present on 10–24% of Indo-

Fijian sugarcane farms in western Viti Levu, Fiji (Thaman and Ali 1993). In comparison, mango (*Mangifera indica*), papaya (*Carica papaya*), drumstick tree (*Moringa oleifera*), *Murraya koenigii*, and tamarind (*Tamarindus indica*) were found on 75–100% of the farms. In Hawai‘i, it is occasionally found in homegardens, and it is sold in farmer’s markets, although commercial production is minor. Jackfruit is occasionally planted in backyard gardens in Guam. The species is also reported to have been introduced to Palau, Yap, Pohnpei, Nauru, Tabiteuea in Kiribati, Samoa, and other islands (Fosberg et al. 1979).

1.28 Botanical Description

1.28.1 Preferred scientific name

Artocarpus heterophyllus Lam.

1.28.2 Family

Moraceae (mulberry family)

1.28.3 Non-preferred scientific names

Artocarpus brasiliensis Gomez

Artocarpus heterophylla Lam.

Artocarpus maxima Blanco

Artocarpus philippinensis Lam.

Polyphema jaca Lour.

Soccus arboreus major Rumph.

Artocarpus integer (Thunb.) Merr and its synonym *A. integrifolia* L. f. are a different species (champedak), and these names have often mistakenly been used as synonyms for *A. heterophyllus*.

1.28.3 Common names

Pacific islands *dapanapan*(?) (Yap)

jack, jack tree, jackfruit, jak, jakfruit (English) *jacquier* (French)

kapiak (Papua New Guinea)
uto ni India (Fiji)
'ulu initia (Samoa)
Other regions
banun, *khanun*, *makmi* (Thai)
buen pan, *jaca*, *pan de fruta*, *rima* (Spanish)
chakki, *kanthal*, *kathal*, *kathar*, *panos* (Hindi)
Jackfrutchbaum (German)
langka, *nancas* (Filipino)
nangka, *nongko* (Javanese)
Species Profiles for Pacific Island Agroforestry (www.traditionaltree.org)

1.28.4 Size and form

Jackfruit is a medium-size, evergreen tree that typically attains a height of 8–25 m (26–82 ft) and a stem diameter of 30–80 cm (12–32 in). The canopy shape is usually conical or pyramidal in young trees and becomes spreading and domed in older trees. The canopy diameter at 5 years old
ranges from 3.5–6.7 m (11–22 ft) and can reach 10 m or more in older trees. The tree casts a very dense shade. Heavy side branching usually begins near the ground. All parts of the tree exude a sticky white latex when injured.

1.28.5 Flowers

This species is monoecious, having male and female inflorescences (or "spikes") on the same tree. Male and female spikes are borne separately on short, stout stems that sprout from older branches and the trunk. Male spikes are found on younger branches above female spikes. Male spikes are dense, fleshy, cylindrical to club-shaped, and up to 10 cm (4 in) in length. Flowers are tiny, pale green when young, turning darker with age. Female flowers are larger, elliptic or rounded, with a tubular calyx. The flowers are reportedly pollinated by insects and wind, with a high percentage of cross-pollination.

1.28.6 Leaves

Leaves are dark green, alternate, entire, simple, glossy, leathery, stiff, large (up to 16 cm [6 in] in length), and elliptic to oval in form. Leaves are often deeply lobed when juvenile and on young shoots.

1.28.7 Fruit

Jackfruit has a compound or multiple fruit (syncarp) with a green to yellow-brown exterior rind that is composed of hexagonal, bluntly conical carpel apices that cover a thick, rubbery, whitish to yellowish wall. The acid to sweetish (when ripe) banana-flavored flesh (aril) surrounds each seed. The heavy fruit is held together by a central fibrous core. Fruits are oblong-cylindric in shape, typically 30–40

1.28.8 Left: Female (top) and male (bottom) flower spikes. Right: Seedlings have lobed leaves compared to the entire leaves on mature trees. photos: C. Elevitch *Acrocarpus heterophyllus* (jackfruit) cm (12–16 in) in length but sometimes up to 90 cm (35 in). They usually weigh 4.5–30 kg (10–66 lb), although a weight of 50 kg (110 lb) has been reported (Morton 1987). The heavy fruit is borne primarily on the trunk and interior part of main branches. Fruits take 90–180 days to reach maturity. In the Northern Hemisphere, the main bearing season is late spring to early fall (between March and September). A few fruits mature in winter or early spring.

1.28.9 Seeds

Seeds are light brown to brown, rounded, 2–3 cm (0.8–1.2 in) in length by 1–1.5 cm (0.4–0.6 in) in diameter, and enclosed in a thin, whitish membrane. Up to 500 seeds can be found in each fruit. Seeds are recalcitrant and can be stored up to a month in cool, humid conditions.

1.28.10 Rooting habit

Jackfruit has a strong taproot.

1.28.11 Similar species

Champedak (*Artocarpus integer* [Thunb.] Merr.) is easily mistaken for jackfruit. There are several indicators differentiating the two species; perhaps the easiest to see is that champedak has smaller, rounder fruits, with less latex and thicker rind. However, champedak is rarely found in the Pacific.

Hyperlipidaemia mainly increased level of cholesterol or low-density

lipoprotein cholesterol (LDL-C) contributes significantly to the manifestation and development of atherosclerosis and coronary heart diseases (CHDs). Cardiovascular diseases, including atherosclerosis, are the most common causes of mortality and morbidity worldwide. Approximately 12 million people reportedly die of cardiovascular disease each year worldwide. Although several factors such as diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle play a significant role in causing heart failure, the high level of cholesterol, particularly LDL-C is mainly responsible for the onset of CHDs. The lowering of lipids and cholesterol levels by drug or dietary interventions could reduce the risk of CHDs. The known lipid-lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but they also have many side effects.Therefore, the development of lipid-lowering drugs from natural sources is the best option and is in great demand. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems.

Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of hyperlipidaemias in many parts of the world. There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine**.** Recently, the search for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs. Plants that were once considered of no value are now being investigated, evaluated and developed in to drugs with no side effects. One of such plant is *S. hispida*.Linn commonly known as ‘Shaggi button weed’ belongs to the family *Rubiaceae* and is widely distributed throughout the world as a useful medicinal plant . The seeds of plants as confection are cooling demulcent and given in diarrhea and dysentery. Seeds have been recommended as a substitute for coffee. Seeds are crushed in to paste and taken orally to treat stomach problems. According to some studies, *S. hispida*.Linn has also anti hypertensive activity. The plant has been

extensively studied for its phytochemical composition and a large number of active ingredients such as, Borreline, β-sitosterol, Ursolic acid and Isorhmnatin. Recently, pharmacological studies have shown that *S. hispida* seeds exhibit antidiabetic properties in rats. Hence, in the present study, the ethanolic extract of *S. hispida* seeds was investigated for Antihyperlipidaemic activity in triton WR-1339 induced hyperlipidaemic rats.

CHAPTER II

2. LITERATURE REVIEW

***S. Chackrewarthy et al*.(2002)**[22], in his study investigates the hypoglycemic and hypolipidemic effects of an ethylacetate (EA) fraction of the mature leaves of *A. heterophyllus* in a streptozotocin (STZ) induced diabetic rat model. In normoglycemic rats, administration of a single dose (20 mg/kg) of the EA fraction resulted in a significant ($P < 0.05$) reduction in the fasting blood glucose concentration and a significant improvement in glucose tolerance ($P < 0.05$), compared to the controls. In STZ-induced diabetic rats, chronic administration of the EA fraction of *A. heterophyllus* leaves daily for 5 weeks resulted in a significant lowering of serum glucose, cholesterol and triglyceride (TG) levels. Compared to control diabetic rats, the extract-treated rats had 39% less serum glucose, 23% lower serum total cholesterol and 40% lower serum TG levels and 11% higher body weight at the end of the fifth week.

***Haidy S. Omar et al*.,(2013)[22]** examined the antioxidative, hypoglycemic, and hypolipidemic activities of Artocarpusheterophyllus (jack fruit) leaf extracts. Various extracts like 70% ethanol n-butanol, water, chloroform, and ethyl acetate extracts are examined. The administration of 70% ethanol extract or n-butanol extract to streptozotocin (STZ)-diabetic rats significantly reduced fasting blood glucose (FBG) from 200 to 56 and 79 mg%, respectively; elevated insulin from 10.8 to 19.5 and 15.1 µU/ml, respectively; decreased lipid peroxides from 7.3 to 5.4 and 5.9 nmol/ml, respectively; decreased %glycosylated hemoglobin A1C (%HbA1C) from 6.8 to 4.5 and 5.0%, respectively; and increased total protein content from 2.5 to 6.3 and 5.7 mg%, respectively. Triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), VLDL-C, and LDL/HDL ratio significantly declined by -37, -19, -23, -37, and -39%, respectively, in the case of 70% ethanol extract; and by -31, -14, -17, -31, and -25%, respectively, in the case of n-butanol extract; as compared to diabetic rats. HDL-C increased by +37% (70% ethanol extract) and by +11% (n-butanol extract). Both JFEE and JFBE have shown appreciable results in decreasing FBG, lipid peroxides, %HbA1C, TC, LDL-C, and TG levels, and increasing insulin, HDL-C, and protein content.

Venkateswarulu .M et al.,(2007)[22] in his study used aqueous extract from Artocarpusheterophyllus leaves to evaluated for its hypocholesterolaemic and hypotriglyceridemic activities. The animals were divided into Normal (CG), Triton treated group (T), Triton plus Atorvastatin, Triton plus herb extract 200 mg/kg, Triton plus herb extract 400 mg/kg, treated groups. Oral administration of Artocarpusheterophyllus leaf extract (200 mg/kg and 400 mg/kg) in both groups At 24 hrs after treatment with TRITON WR 1339 caused a significant decrease in serum lipid parameters like Triglycerides (TG), Cholesterol (CH), LDL- cholesterol, Atherogenic index (AI), LDL/HDL Ratio and Total proteins as like in atorvastatin treated groups. The both extract treated groups and atorvastatin treated group bought about a significant increase in HDL-Cholesterol levels levels.

K Periyanayagam et al.,(2012)[23] *studied the* wound healing activity of the leaves of *artocarpusheterophyllus lam*. (moraceae) on ex-vivo porcine skin

wound healing model and found that that the ethyl acetate extract of the leaves possesses potential wound healing activity.

P. Sivagnanasundaram et al.,(2010)[22] investigated and evaluated the antimicrobial and phytochemical properties of *Artocarpusheterophyllus* in leaf and stem bark extracts Hexane, dichloromethane and ethanol were used as extraction solvents and test organisms were Escherichia coli, Micrococcus luteus, Aspergillusniger and Trichoderma sp. A disc diffusion test was adopted to test the susceptibility of the selected microbes to the extracts while Minimum inhibitory concentration (MIC) was determined using serial dilution of extracts. Ethanolic stem bark extracts (30mg/ml) of A.heterophyllusexibits significant antibacterial activity against Escherichia coli with 9.50 ± 0.44 inhibition zone radii. Dichloromethane extracts of leaf and stem bark showed lesser antibacterial activity against both of the bacteria with inhibition zones of 3.00 ± 0.34 mm to 5.66 ± 0.16 mm while hexane extracts did not show any antibacterial activity. Antifungal activity on the other hand was not detected in any of the extracts. Phytochemical screening confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes.

E. R. Suchithra et al.,(2006)[22] in his study "Antidiabetic activity of Artocarpusheterophyllus rag extract studied in high fat fed- low dose STZ induced experimental type 2 diabetic rats" reports that *Artocarpusheterophyllus rag* possess antibacterial, anti-inflammatory, antioxidant and immunomodulatory properties. In the study Diabetic rats were treated with Artocarpusheterophyllus rag extract at a dosage of 300 mg/kg b.w daily for 30 days. Metformin (200 mg/kg. b.w) was used as a reference drug and fasting blood glucose, plasma insulin and HbA1c were the parameters under consideration. The extract supplementation attenuated the elevated levels of glucose, glycosylated hemoglobin, AST, ALT and ALP. The insulin level was improved with an improvement in hepatic glycogen content of insulin resistant diabetic rats. The altered activities of glycogen metabolizing enzymes were normalized upon extract treatment. Also the extract improves insulin sensitivity which is evident from intraperitoneal insulin

tolerance test. The results show that the rags of Artocarpusheterophyllus is non-toxic and possess significant antidiabetic properties.

Praveen et al.,(2007)[22] herbal remedies have evolved with enormous impending of alleviate. Herbal medicine progress against the non-communicable disease like diabetes is one of the propel area of research in the field of worldwide medicine. Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the assortment of lipid and lipoprotein fractions. Hyperlipidemia has been one of the maximum risk factors contributing to the occurrence and relentlessness of coronary heart diseases. HMG Co A reductase is a key enzyme involving in rate limiting step of cholesterol biosynthesis. Conservative anti-hyperlipidemic drugs have restricted efficacies and vital side effects, so that alternative lipid lowering agents are required. This review explains the plants possessing significant anti-hyperlipidemic activity with their botanical name, family, part used, extract used and inducing agent of hyperlipidemia

Sivaganasundaram et al.,(2008)[23] Increasing drug resistance of pathogens and negative consequences of antibiotic usage has led to the search for alternative medicines from nature. Many plants have been exploited to cure infectious diseases from time immemorial. The present investigation evaluated the antimicrobial and phytochemical properties of *Artocarpus heterophyllus i.e.* Jack fruit (Kos in Sinhala) and *Artocarpus altilis i.e.* Bread fruit (Dhel in Sinhala) leaf and stem bark extracts. Hexane, dichloromethane and ethanol were used as extraction solvents and test organisms were *Escherichia coli*, *Micrococcus luteus*, *Aspergillus niger* and *Trichoderma* sp. A disc diffusion test was adopted to test the susceptibility of the selected microbes to the extracts while Minimum inhibitory concentration (MIC) was determined using serial dilution of extracts. Phytochemical screening was carried out by specific chemical identification tests. Bioassay data were statistically analyzed using two-way ANOVA (SPSS 20 at 95% confidence level). Ethanolic stem bark extracts (30mg/ml) of *A.heterophyllus* and *A.altilis*

possessed significant antibacterial activity against *Escherichia coli* with 9.50 ± 0.44 mm and 7.49 ± 0.28 mm inhibition zone radii respectively. Dichloromethane extracts of leaf and stem bark showed lesser antibacterial activity against both of the bacteria with inhibition zones of 3.00 ± 0.34 mm to 5.66 ± 0.16 mm while hexane extracts did not show any antibacterial activity. Antifungal activity on the other hand was not detected in any of the extracts. Bacterial antibiotic tetracycline and fungal antibiotic ketoconazole which were used as positive controls were more effective even at 1/10 concentration compared to all the plant extracts tested. Phytochemical screening confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes in both of the trees. These results confirm the potential antibacterial activity of *A.heterophyllus* and *A.altilis*

Haidy et al.,(2005)[22] The present study examines the antioxidative, hypoglycemic, and hypolipidemicactivities of *Artocarpus heterophyllus* (jack fruit) leaf extracts (JFEs). The 70% ethanol (JFEE), n-butanol (JFBE), water (JFWE), chloroform (JFCE), and ethyl acetate (JFEAE) extracts were obtained. Both JFEE and JFBE markedly scavenge diphenylpicrylhydrazylradical and chelate Fe+2 *in vitro*. A compound was isolated from JFBE and identifiedusing 1D and 2D 1H- and 13C-NMR. The administration of JFEE or JFBE to Streptozotocin (STZ)-diabetic rats significantly reduced fasting blood glucose (FBG) from 200 to 56 and 79 mg%, respectively; elevated insulin from 10.8 to 19.5 and 15.1 μU/ml, respectively; decreased lipid peroxides from 7.3 to 5.4 and 5.9 nmol/ml, respectively; decreased %glycosylated hemoglobin A1C (%HbA1C) from 6.8 to 4.5 and 5.0%, respectively; and increased total protein content from 2.5 to 6.3 and 5.7 mg%, respectively. Triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), VLDL-C, and LDL/HDL ratio significantly declined by -37, -19, -23, -37, and -39%, respectively, in the case of JFEE; and by -31, -14, -17, -31, and -25%, respectively, in the case of JFBE; as compared to diabetic rats. HDL-C increased by +37% (JFEE) and by +11% (JFBE). Both JFEE and JFBE have shown appreciable results in decreasing FBG, lipid peroxides, %HbA1C, TC, LDL-C, and TG levels, and increasing insulin, HDL-C, and protein content. The spectrometric analysis

confirmed that the flavonoid isolated from JFBE was isoquercitrin. We can conclude from this study that JFEE and JFBE exert hypoglycemic and hypolipidemic effects in STZ-diabetic rats through an antioxidative pathway that might be referred to their flavonoid contents.

Boopathy raja et al.,(2004)[23] Herbal medicines have good curative effect on certain diseases especially for diabetes mellitus which needs continuous medication throughout the life. Present day allopathic medicines are costlier and having more side effects which could cause severe damages to the vital organs. Hence, finding a suitable herbal medicine for diabetes mellitus is very important in the current situation. In this present study, the fruit extract of Helicteres isora was used to evaluate the antihyperlipidemic activity in streptozotocin induced diabetic rats. Powdered fruits of Helicteres isora were extracted in ethanol and the crude extract was used for the treatment of diabetic rats. Streptozotocin was used to induce the diabetic condition in wistar rats. For the treatment, the drug glibenclamide also used to treat the diabetic rats to compare the efficacy of the herbal extract. After 45 days of treatment, the animals were sacrificed and lipid profiles were estimated in the serum and liver. The serum and liver lipid levels were abnormal in streptozotocin induced diabetic rats than in the control rats. Total cholesterol, triglycerides, phospholipids, LDL and VLDL were elevated and the HDL level was significantly decreased in diabetic rats. After treated with Helicteres isora fruit extract (HiFE), the lipid levels of diabetic rats were restored to near normal level. HiFE has the potential of antihyperlipidemic activity which was proved by the above results. It is suggested that HiFE may have the similar action mechanism of glibenclamide.

Bramha et al.,(2002)[22] The anti-hyperlipidemic effect of methanolic extract of whole plant of *Rhinacanthus nasutus* ((RNM) was tested in Triton and fat diet induced hyperlipidemic rat models. Here, Acute hyperlipidemia was induced by administration of single dose of Triton X 100 (400 mg/kg,i.p) and Chronic hyperlipidemia was induced by feeding fat diet for 21 days to rats. Treatment

with RNM (200 and 400 mg/kg, p.o) significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) , and increase of serum High Density Lipoprotein Cholesterol (HDL-C) when

compared to vehicle control and standard drug Atorvastatin (10 mg/kg). The results demonstrated that methanolic extract of whole plant of *Rhinacanthus nasutus* possessed significant antihyperlipidemic activity.

Syed et al.,(2000)[26] designed to perform preliminary phytochemical screening, acute oral toxicity and to evaluate antihyperglycemic activity of whole plant of *Glycosmis pentaphylla* ethanolic extract. .*Glycosmis pentaphylla,*whole plant was extracted using ethanol as solvent by soxhlet apparatus. The extract was subjected to preliminary phytochemical screening. Acute oral toxicity studies were performed to determine test dose .The evaluation of antihyperlipidemic activity was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, and amino acids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. Cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were measured. The results suggested that EGP (ethanolic extract.*Glycosmis pentaphylla*) possess antihyperlipidemic activity against hyperlipidemia induced by Triton X 100 and also High Fat Diet induced experimental models.

Sivaelango et al.,(2014)[26] Hyperlipidaemia is the greatest risk factor of coronary heart disease. Currently available hypolipidaemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidaemia has no side effects and is relatively cheap and locally available. Literature claims that Saponins are able to reduce hyperlipidemia. Based on high saponin content in herbal plants, *Spermacoce hispida* (*S. hispida*) was selected and the present study focus on the antihyperlipidaemic activity of

ethanolic seed extract of *S. hispida* against triton-WR-1339 induced hyperlipidaemia in rats. Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. *S. hispida* was administered orally at a dose of 200 mg/kg to triton WR-1339 induced hyperlipidaemic rats. After administration of *S. hispida* shows a significant decrease in the levels of cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of HDL in serum and liver tissues against triton induced hyperlipidaemic in rats. Therefore it effectively suppressed the triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

Nazli shahin et al.,(2013)[28] Phytochemical investigation of the leaves of *Artocarpus heterophyllus* furnished six compounds from different combinations of petroleum ether, chloroform and methanol. Structures of these compounds were elucidated and established by standard spectroscopic methods. Isolated compounds are n-Octadec-9-enoyl α-L-rhamnopyranoside(1), n-octadec-9,12-dienoyl-α-L-rhamnopyranoside (2), n-octadec-9,12-dienoyl-β-D-glucopyranoside (3), n-octadec-9-enoyl-β-D-glucopyranoside (4), n-octadec-9-enoyl-β-D-arabinopyranoside (5) and n-octadec-9-enoyl-α-D-xylopyranoside (6) respectively. The structures of all the phytoconstituents are elucidated on the basis of spectral data analyses and chemical reactions.

Khyati et al.,(2014)[20] studied Mangifera indica L., known as mango (Family; Anacardiaceae), commonly used herb in ayurvedic medicine, traditionally used for their antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties, antibacterial, antifungal, anthelmintic, antiparasitic, antitumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, antimicrobial, hepatoprotective, gastroprotective effects.To investigate effect of aqueous extract of Mangifera indica L.leaf on high cholesterol fed diet rats. High cholesterol fed diet rats exhibited significant increase in total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and significant decrease in high density lipoproteins. Treatment with aqueous

extract of Mangifera indica leaves significantly decreased total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and increased in high density lipoproteins rats. Hypolipidemic activity of M. indica may be attributed due to presence of flavonoids, Saponins, glycosides, tannins, phenolics.

Alma et al.,(2008)[27] Hyperlipidemia plays an important role in the development of atherosclerosis, the main cause of death in the world. In this study, the lipid-lowering effect of *Carica papaya* leaf in rats fed with a high cholesterol diet was evaluated. Daily doses of *C. papaya* extract 0, 31, 62 or 125 mg/kg body weight were orally administered in 300 µl polyethylene glycol to hypercholesterolemic rats; it was also administered 62 mg/kg body weight of the extract to rats with normal diet. After a 20-day treatment, the animals were sacrificed; blood and liver were analyzed. Hypercholesterolemic rats showed an increased serum and liver cholesterol, triacylglycerols, and atherogenic index. The *C. papaya* extract produced a significant decrease of serum and liver cholesterol concentrations in hypercholesterolemic rats, but did not modify serum or liver triacylglycerols; however, the extract reduced the atherogenic index in a dose-dependent manner. *C. papaya* treatment decreased LDL-C and increased HDL-C in serum significantly. When the oxygen consumption was evaluated in phosphorylating and resting states, the respiratory control in hypercholesterolemic rats mitochondria was lower than in normal diet rats. However, a higher respiratory control in hypercho-lesterolemic rats mitochondria was observed after C papaya treatment. The liver morphological data are in accordance with serum and liver biochemical values. Our data support that *C. papaya* has a significant hypocholesterolemic action and HDL-C raising effect on rats fed with a cholesterol- rich diet, however, the precise metabolites responsible of this effect remain unknown.

CHAPTER III

3. AIM AND OBJECTIVE

3.1 Aim

To successfully evaluate the antihyperlipidemic and antioxidant activity of *Artocarpusheterophyllus stem extract on* High fat diet-induced hypercholesterolemia and triton induced hyperlipidaemia models.

3.2 Objective

1. To conduct a literature survey for establishing the relavence of the study.
2. To Collection and authenticate of *Artocarpusheterophyllus* stem.

3. To successfully extract the dried stem of *Artocarpusheterophyllus using* suitable solvents.
4. To evaluate toxicological profile of the extract.
5. To characterize the antioxidant property of the extract.

CHAPTER IV

4. PLAN OF THE WORK

4.1 Plan Of The Work

- Plant collection
- Plant authentication
- Solvent extraction
- Phytochemical screening
- Acute toxicological studies

- Pharmacological screening for antihyperlipidaemic activity
    - High-cholesterol diet induced rat model.
    - Triton induced rat model.
    - Effect on Normocholesteremic rats

4.2 Pharmacological screening for antihyperlipidaemic activity

1. Superoxide radical scavenging activity
2. DPPH radical reducing activity
3. Lipid peroxidation assay
4. Nitric oxide scavenging assay
5. Hydrogen per oxide assay

CHAPTER V

5. PLANT PROFILE OF ARTOCARPUS HETEROPHYLLUS

Table No 3. General characters of *Artocarpus Heterophyllus*

Symbol	*ARHE2*
Group	*Dicot*
Family	*Moraceae*
Duration	*Perennial*
Growth Habit	*Tree*

Symbol	**Scientific Name**
ARIN18	*Artocarpus integer* auct.
ARIN20	*Artocarpus integrifolius* auct.

Rank	**Scientific Name and Common Name**
Kingdom	*Plantae – Plants*
Subkingdom	*Tracheobionta – Vascular plants*
Superdivision	*Spermatophyta – Seed plants*
Division	*Magnoliophyta – Flowering plants*
Class	*Magnoliopsida – Dicotyledons*
Subclass	*Hamamelididae*
Order	*Urticales*
Family	*Moraceae – Mulberry family*
Genus	Artocarpus *J.R. Forst. & G. Forst. – breadfruit*
Species	Artocarpus heterophyllus *Lam. – jackfruit*

Fig No 1. Fruits of ARTOCARPUS HETEROPHYLLUS

Fig No 2. Whole parts of ARTOCARPUS HETEROPHYLLUS-I

Fig No 3. Whole parts of ARTOCARPUS HETEROPHYLLUS-II

Fig No 4. Whole parts of ARTOCARPUS HETEROPHYLLUS-III

Fig No 5. Whole parts of ARTOCARPUS HETEROPHYLLUS-IV

Fig No 6. Whole parts of ARTOCARPUS HETEROPHYLLUS-V

Fig No 7. Whole parts of ARTOCARPUS HETEROPHYLLUS-VI

Fig No 8. Whole parts of ARTOCARPUS HETEROPHYLLUS-VII

Fig No 9. Whole parts of ARTOCARPUS HETEROPHYLLUS-VIII

CHAPTER VI

6.MATERIALS AND METHODS

6.1 Solvent Extraction And Phytochemical Screening

Plant collection and extraction Artocarpus heterophyllus were collected from the regions of ananthagiri hills vikarabad, Telengana. After that the plant parts such as leaf and bark were coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

Phytochemical screening Qualitative phytochemical screening with the extract of both the plants Artocarpus heterophyllus was determined as follows: Carbohydrates(Anthrone method),Alkaloids(200 mg plant material in 10 ml methanol ,filtered); a 2ml filtrate + 1%HCL + steam,1 ml filtrate+6 drops of Mayor, s reagent/Wagner, s reagent/Dragendroff reagent,creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Flavanoids (200 mg plant material in 10 ml ethanol, a 2 ml filtrate + conc. HCL+ magnesium ribbon pinktomato red colour indicated the presence of falvanoids.Tannins, (200 mg plant material in 10 ml distilled water , filtered): a 2ml filtrate + 2 ml FeCl3 , blue black precipitate indicated the presence of tannins. Glycosides(Keller-Killani test: 2 ml filtrate+ 1 ml glacial acetic acid + FeCl3 + conc.H2SO4); green – blue colour indicted the presence of glycosides. steroids(Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered);a 2ml filtrate +2 ml acetic anhydride +conc.H2SO4. blue ring indicated the presence of terpenoids, Saponins(frothing test: 0.5 ml filtrate+ 5 ml distilled water); frothing persistence indicated presence of saponins. Anthraquinones- 2 ml of plant extracts were treated with 1 ml of dilute ammonia and shaken vigorously. Pink red colour in the ammonical layer indicates the presence of anthraquinones. Cardiac glycosides (Keller-Killani test) were analysed. Anti microbial screening were carried out in nutrient agar media.

6.2 Preparation Of *Artocarpus Heterophyllus* Stem Extract

The barks were initially separated from the main tree and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. Calculated amount of powder in contrasting amount of organic solvents in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. And then it was filtered with the help of muslin cloth and centrifuged. The supernatant was collected and the solvent was evaporated by solvent distillation apparatus to make the final volume of one-fourth of the original volume. It was stored at 40 °C in air tight bottles for further studies.

6.3 Qualitative Phytochemical Screening Of Plant Extracts

The crude extracts of the plant were subjected to chemical tests for the identification of various active constituents as described below.

Table No 4. Qualitative test protocol

S. No	Test	Methods Used
1	Alkaloids	Dragendroff's test, Wagner's test, Mayer's test
2	Flavanoid	Sulphuric acid test
3	Steroids	Liebermann Burchard test, Salkowaski test
4	Saponins	Foam Test
5	Phenolic Compounds & Tannins	Bromine Water Test
6	Fats &Oils	Sudan Red III reagent Test

7	Glycosides	Keller-killiani Test
8	Protein	Biuret test, Xanthophoretic test, Lead acetate test
9	Carbohydrates	Molisch test, Fehling test, Benedict's test
10	Amino Acids	Ninhydrin test

6.4 Acute Toxicity Studies

6.4.1 Animals

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±5°C with light dark cycle of 12 h in the Animal House of the study center are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) (KU/IAEC/M.Pharm/174) and animal care wastaken as per the guidelines of Committee for the Purpose of Controland Supervision of Experiments on Animals (CPCSEA), Government of India.

6.5 Study Design

6.5.1 Acute Toxicological Studies

Acute toxicity studies for the extracts were conducted as per OECD guidelines 423 using Albino mice of both sex (20 -25gm). Each animal was administered the extracts by oral route. The animals were observed for mortality up to 72 hrs. The ethanolic and aqueous extract was found to be safe up to 2000 mg/kg body weight.

6.5.2 High – Cholesterol Diet Model

Swiss albino rats weighing (200 – 250gm) were used for a standard experimental method as high cholesterol diet consisting of Cholesterol (1%),

sodium cholate (0.5%), sucrose (30%), casein(10%), butter (5%) and standard chow diet (53.5%) for 7 days. The animals divided into three groups of control, test and standard drug treated animals. The studies conducted in two stages. In the preliminary stage effective hypolipedemic doses of test and standard drugs are worked out and in the final stage the effect of test and standard drugs are studied. The lipid profile includes total cholesterol LDL, HDL, VLDL and triglycerides were studied. The blood samples were collected after 6, 24 and 48hour of drug administration.

Group 1: Control

Group 2: Ethanolic extract

Group 3: Aqueous extract

Group 4: Standard Fenofibrate

6.5.3 Triton Induced Hyperlipidemic Rats

The antihyperlipidemic effects of the above extracts were evaluated in 45 triton induced hyperlipidemic rats starved for 18hours. The rats were divided into 4 Groups of 5 animals each. and then injected with Triton at a dose of 100mg/kg body weight except rats of group 1 which served as a normal vehicle treated group 2 and 3 were treated daily with a dose of 200 and 400mg/kg ethanolic and aqueous extracts respectively immediately after the Triton injection by i.p. administration. Blood samples were collected after 6, 24 and 48 hour of Triton injection evaluates the lipid profiles.

Group 1: Control

Group 2: Ethanolic extract

Group 3: Aqueous extract

Group 4: Standard Fenofibrate

6.5.4 Effect on Normocholesteremic Rats

The hypolipedemic effects of the extracts were evaluated in 4 groups of Normocholesteremic rats fasted for 18hour and these studies were carried out as described for antihyperlipidemic effects the rats were treated orally for 7 days with the divided doses of 200mg/kg and 400mg/kg p.o. After the end of the stipulated period of drug treatment, all animals were starved for 20hour and blood samples were collected from the puncture of retro-orbital plexus and analysed for blood lipid profile.

Group 1: Control

Group 2: Ethanolic extract

Group 4: Standard Fenofibrate

6.5.5 Biochemical Analysis of Serum

Serum samples were analysed for total cholesterol, High density lipoproteins, Low density lipoproteins and very low density lipoproteins using standard enzymatic assay kit.

6.6 Acute Toxicity

The toxicity for the aqueous and ethanolic extracts stem of Artocarpus heterophyllus was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPSEA was adopted for toxicity studies.

6.7 In Vitro Antioxidant Activities

6.7.1 Superoxide Radical Scavenging Activity

6.7.1.1 Principle

The assay is based on the ability of drug to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of riboflavin within the system. The superoxide radical thus generated reduce the NBT to a blue colored complex.

6.7.1.2 Reagents

- Nitro blue tetrazolium (NBT) - 1.5nm (12.3mg/10ml)
- Riboflavin - 0.12µm (4.5mg/100ml)
- NaCN/EDTA - 0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

6.7.1.3 Procedure

The reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, Riboflavin (0.12mM), NBT (1.5 n moles), Phosphate buffer (67mM, pH 7.8) and various concentrations of the flower extract in a final volume of 3ml. The tubes were illuminated under incandescent lamp for 15min. The optical density at 560 nm was measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of seed oil extract and fraction-IV. Vitamin C was used as positive control. The concentration of fraction-IV required to scavenge 50% superoxide anion (IC_{50} value) was then calculated.

Calculation

$$\% \text{ inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

6.8 Dpph Radical Reducing Activity

6.8.1 Principle

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radical, DPPH (2, 2- Diphenyl - 1- picrylhydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stochiometric with respect to the number of electrons captured.

6.8.2 Reagents

- DPPH - 3mg in 25ml methanol (stored in dark bottle)
- Methanol

6.8.3 Procedure

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of seed oil extract and fraction-IV. The volume was made up to 1ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

6.9 Lipid Peroxidation Activity

- The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.
- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final

concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.

- Potassium hydrogen phosphate (0.19 gram) was mixed with 8 gm of sodium chloride. To this 2.38 gm of disodium hydrogen phosphate was dissolved and made up to 1000 milliliter alongside DM H_2O and pH was adjusted to 7.4
- To a set of eight clean dry test tubes, 2 ml of 0.25Mm HCL containing 15% trichloroacetic acid and 0.38% thiobarbituric acid were added and to this 1 ml of different concentrations of the test extracts were added. For five minutes the sample was kept. Centrifugation was done and absorbance of the upper layer was measured at 538 nm and the lipid peroxide content was found. All experiments were performed in triplicate.

6.10 Nitric Oxide Scavenging Method

- The stocking solutions, various working conc. were produced to avail concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.
- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.
- Sodium nitroprusside 5mM in phosphate buffer at pH 7.4 saline was added with a range of concentrations of the test sample or standard and incubated at 25^{o}C for 150 minutes. At regular intervals, 1.5 ml of samples (incubated test sample) were taken off and a poured with 1.5 ml Griess reagent (1% Sulphanilamide, phosphoric acid (2 percent), and 0.1 percent NEDA 2.HCL.
- The absorbance was read at 540 nm. The difference in the absorbance between test and control on nitric oxide was determined and depicted as percent scavenging of NO radical.

- Capability to scavenge the NO radical was designed by using standard formula .All experiments were performed in triplicate.

6.11 Hydrogen Peroxide Method

- The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water.
- The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.
- 1 ml of standard and test solution was added to 0.6 ml hydrogen peroxide solution. After 10 minutes the reading of the solution was read at 230 nanometer using UV/VIS spectrophotometer alongside a blank containing PBS without H_2O_2.
- The percentage scavenging of hydrogen peroxide of both plant fraction and standard compound were determined.
- The percentage inhibition was calculated to tests & standard making usage of the following formula. All experiments were performed in triplicate.

Calculation

$$\textbf{\% inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

6.12 Triton Induced Rat Model.

6.12.1 Procedure

Eight week old adult male albino rats of *Wistar* strain, weighing approximately 150 to 200 g, were acclimatized for 7 days at room temperature (22±2ºC) and humidity of 45-64% in a 12- hour light/dark cycle in a room under hygienic condition. They were given access to water and a commercial diet *ad libitum*. The experiments were carried out in the Suran labs, Hyderabad, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC).

6.12.2 Chemicals

Triton WR-1339 (A non-ionic detergent, Isooctyl poly oxyethylene phenol) was obtained from Sigma Chemicals Co, Mumbai.

6.12.3 Induction of hyperlipidaemia

Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. After 72 hours of triton injection received a daily dose of 5% CMC in 5ml/kg body weight for 7 days.

6.12.4 Experimental design

In the experiment, the rats were divided into three groups of eight rats each. Group I rats received 5% CMC and considered as controls, Group II rats were treated with Triton WR-1339 (400 mg/kg body weight with Ethanolic extract) and Group III rats were treated with Triton WR-1339 (400 mg/kg body weight with aqueous extract) and ethanolic extract of *Artocarpus heterophyllus* stem (200mg/kg body weight) and Standard fenobirate (100mg/kg body weight).

At the end of 8th day, rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected, and serums were separated by centrifugation. Liver tissues were excised immediately and rinsed in ice-chilled

normal saline, 500mg of the tissues were homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH, 7.4). Biochemical estimations were carried out in serum and liver tissues, parameters such as cholesterol (Zak's, 1977), phospholipids (Rouser *et al*., 1970), triglycerides (Rice, 1970), LDL (Friedwald Levy and Frederickson, 1972), VLDL (Henry *et al*., 1998) and HDL (Varley *et al*., 1980) were analyzed.

6.12.5 Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnnet t test using Graph Pad prism software package 9.05. Results were expressed as mean ± SD from 8 rats in each group. *P* values <0.05 were considered as significant.

CHAPTER VII

7. RESULTS AND DISCUSSION

7.1 Results

The toxicity for the aqueous and ethanolic extracts stem of Artocarpus heterophyllus was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPSEA was adopted for toxicity studies. There were no sign of toxicity for first 48 hours and no animal died on 14 day of study at a dose of 2000 mg/kg.

Table No 5 . Photochemical test

S.No	PHYTOCHEMICAL TEST	EXTRACTS	
		Aqueous	Ethanolic
1	Alkaloids	-	-
2	Tannins	-	+
3	Flavonoids	-	-
4	Steroids	-	+
5	Phenols	+	+
6	Glycosides	+	+
7	Terpenoids	+	+
8	Anthraquinones	-	+
9	Saponins	+	-
10	Caridiac glycosides	-	-

7.2 High-Cholesterol Diet Induced Rat Model

Group 1: animals were fed with a standard diet and was given 0.9% saline once daily for 8 weeks with the aid of oropharyngeal cannula.

Groups 2: animals served as hypercholesterolemic (fed with 2% w/w pure cholesterol enriched diet) negative control.[17]

The animals in group 3, 4 and 5 were fed with 2% w/w pure cholesterol enriched diet supplemented orally with 1 ml of the extract corresponding to 250, 500, and 1000 mg/kg per bwt (LD_{50}>2500.[18]), respectively, once daily for 8 weeks.

Group 6 were fed with 2% w/w pure cholesterol enriched diet and supplemented orally with 1 ml of gemfibrozil (100 mg/kg per bwt) once daily for 8 weeks.

Table No 6. Cholesterol induced diet model

Sample	**Treatment (mg/kg/b.wt)**				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	Standard (GMF)
TC	12.32±0.56	24.67±0.73	15.86±0.98	13.89±0.94	11.44±0.18
TG	8.92±0.13	7.01±19	6.23±0.39	5.99±0.23	4.18±0.37
LDL	4.01±0.27	9.36±0.63	4.05±0.82	4.51±0.37	4.59±0.56
HDL	5.01±0.36	3.03±0.13	3.52±0.55	3.71±0.56	3.89±0.17

HC- High Cholesterol; GMF- Gemfibrozil; TC-total cholesterol; TG-triacylglycerol; LDL- low density lipoprotein; HDL- High density lipoprotein

Table No 7. Blood parameters

Sample	**Treatment (mg/kg/b.wt)**				
Serum	Control	HC	HC+ Ethanolic Extract	HC+ Aqueous Extract	Standard (GMF)
RBC (x 10^6/µL	7.56±0.33	5.64±0.17	6.11±0.34	6.89±0.45	7.34±0.48
Hb (g/dl)	12.6±0.46	8.56±1.76	10.55±0.58	13.22±0.78	13.63±0.56
PCV (%)	38±1.56	29.33±0.77	36.3±1.43	35.89±0.77	36.77±0.34
MCV (pg)	91±0.34	70.33±0.87	90.4±0.47	89.83±0.29	92.38±0.58
MCH (pg)	29.30±0.56	25.14±0.46	26.31±1.87	27.37±0.63	30.62±1.20
MCHC (g/dL)	30.34±0.87	26.95±1.56	30.34±0.56	29.57±0.33	31.56±0.45
WBC (x 10^3/ µL	6.97±1.56	9.97±0.04	9.22±0.56	9.12±0.54	7.59±0.53
Neutrophils (%)	54.36±1.20	34.70±0.42	40.89±0.76	45.35±0.78	50.64±0.74
Monocytes (%)	6.00±2.03	4.99±0.13	5.68±0.57	5.83±0.67	5.43±0.66
Lymphocytes (%)	29.30±0.78	54.0±0.56	48.87±0.56	39.40±0.41	32.67±0.62
Eosinophils (%)	1.90±0.03	2.03±0.32	1.67±0.79	2.05±0.55	1.78±0.21
Basophils (%)	0.24±0.04	0.35±0.23	0.27±0.13	0.25±0.54	0.23±0.50
Platelets (x10^3/µL	325±1.56	647.0±0.45	415.0±0.58	392.0±0.82	341.4±0.16

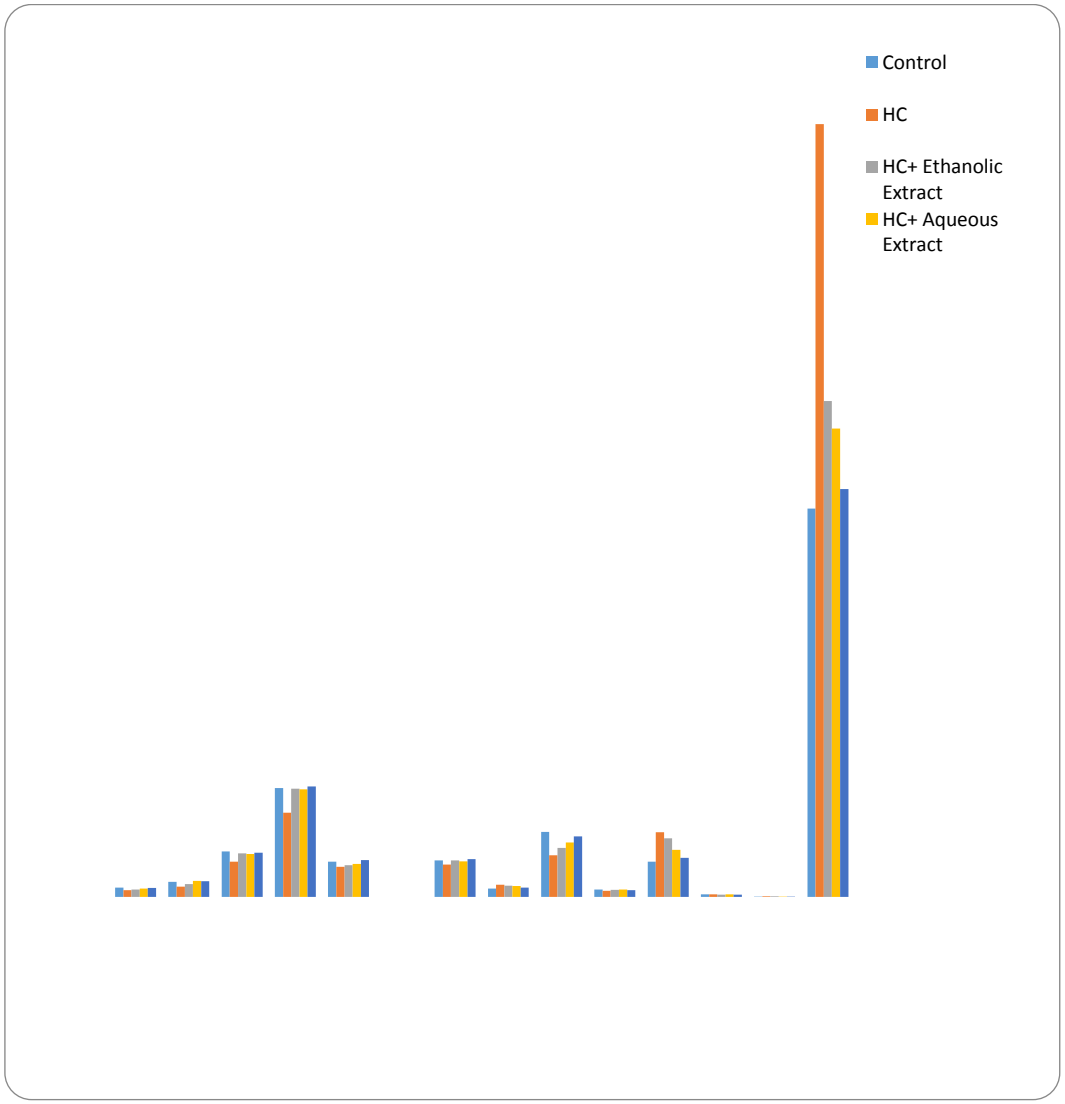

Fig No.10 Scheme of Blood parameters

Table No 8: Effect of *Artocarpus heterophyllus* on changes in the levels of cholesterol and phospholipids in serum and liver tissue of control and experimental animal

Groups	Cholesterol		Phospholipids	
	Serum	Liver	Serum	Liver
Control	077±2.12	062±1.85	050±0.45	085±1.87
Ethanolic extract+Triton	099±0.33	098±0.54	065±0.58	093±0.33
Aqueous extract+ Triton	085±0.87	067±0.33	056±0.67	070±0.76
Standard Fenofibrate	070±0.23	063±1.59	049±1.62	082±1.62

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

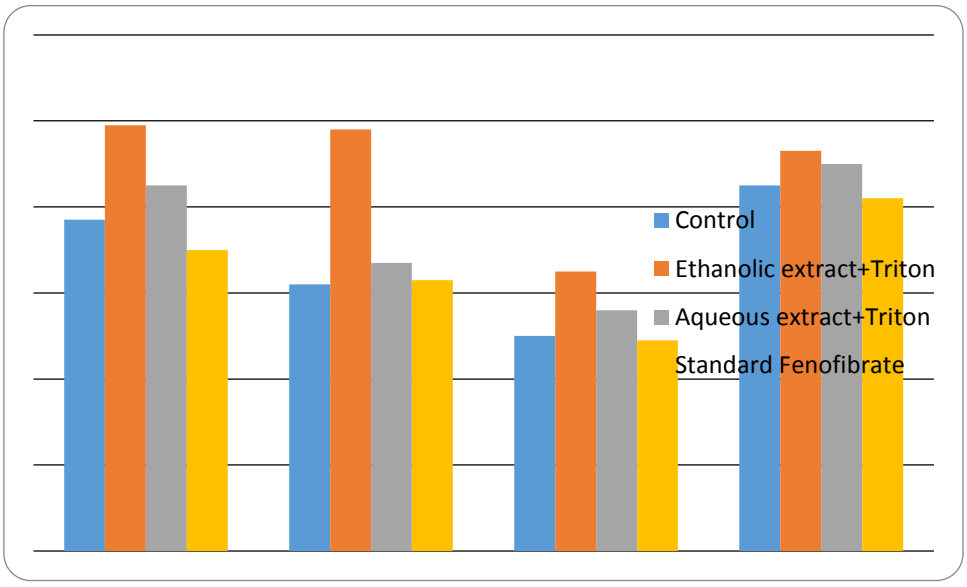

Fig No 11. Scheme of Effects of Cholesterol and Phospholipids

Table No 9: Effect of *Artocarpus heterophyllus* on changes in the levels of triglycerides and LDL in serum and liver tissue of control and experimental animal

Groups	Triglycerides		LDL	
	Serum	Liver	Serum	Liver
Control	075±1.98	061±2.00	038±1.44	021±0.76
Ethanolic extract+Triton	097±0.65	093±0.78	065±0.39	035±0.58
Aqueous extract+Triton	088±0.57	065±1.98	060±1.52	030±1.77
Standard Fenofibrate	069±2.01	057±0.73	035±0.42	019±0.36

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

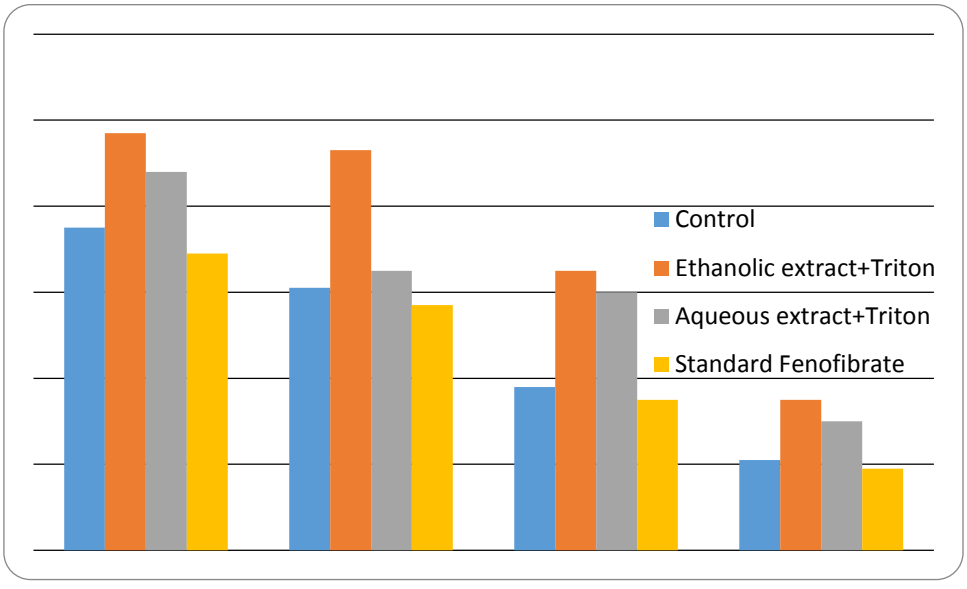

Fig No 12. Scheme of Effects of triglycerides and LDL

Table No 10: Effect of *Artocarpus heterophyllus* on changes in the levels of VLDL and HDL in serum and liver tissue of control and experimental animal

Groups	VLDL		HDL	
	Serum	Liver	Serum	Liver
Control	016±2.60	013±2.05	049±1.62	030±0.24
Ethanolic extract+Triton	015±0.55	014±0.73	045±1.56	026±0.56
Aqueous extract+Triton	014±1.83	011±0.56	035±0.47	020±0.35
Standard Fenofibrate	012±0.92	010±0.37	027±1.83	017±0.28

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

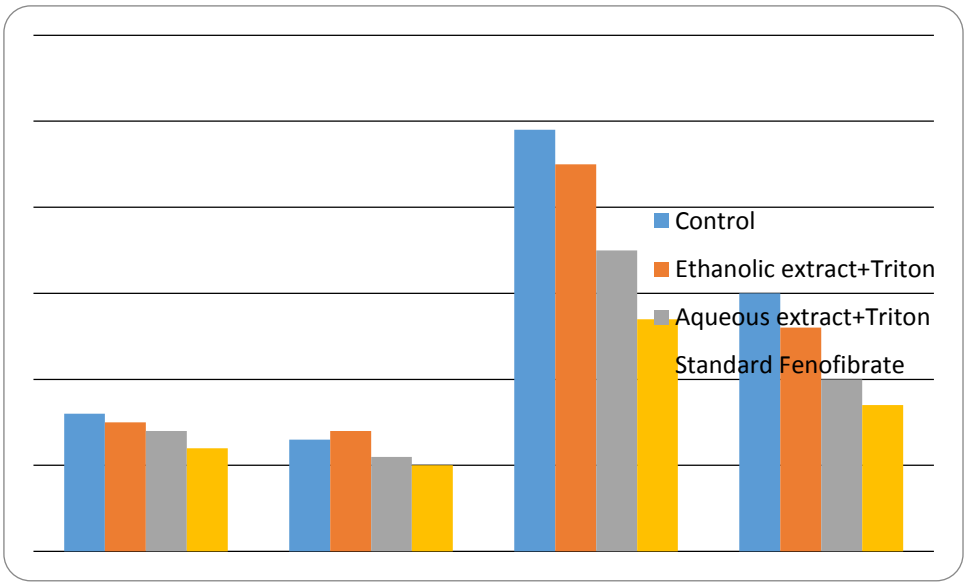

Fig No 13. Scheme of Effects of VLDL and HDL

7.3 Effect On Normocholesteremic Rats

The hypolipidemic effects of the extracts were evaluated in 4 groups fasted for 18 hours and these studies were carried out as described for antihyperlipidemic effects. The rats were treated orally for 7 days. After the end of the stipulated period of drug treatment, all the animals were starved for 20 hours and blood samples were collected from the puncture of retro-orbital plexus and analyzed for blood lipid profile.

Table No 11. Effect of the extracts on blood lipid profile

Groups	Blood lipid profile			
	Cholesterol	**Triglycerides**	**HDL**	**LDL**
Control	75.53±0.35	63.24±1.66	25.76±0.88	60.45±0.23
Ethanolic extract	70.56±0.56	56.73±0.28	29.48±0.22	49.96±0.79
Aqueous extract	64.92±0.73	53.77±0.78	32.15±0.63	41.56±0.69
Standard Fenofibrate	63.23±0.56	50.02±0.45	31.66±0.68	39.56±0.39

In statistical analysis the extract treated groups have been compared with their respective control. $P<0.01$ (ANOVA followed by Dunnett's t-test)

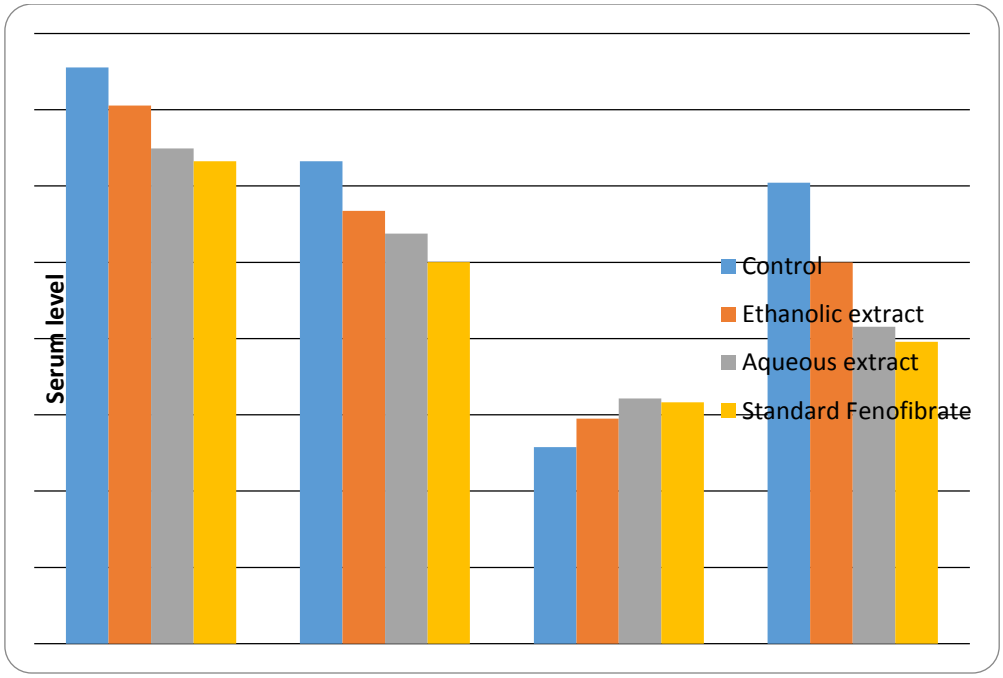

Fig No 14. Scheme of Blood lipid profiles

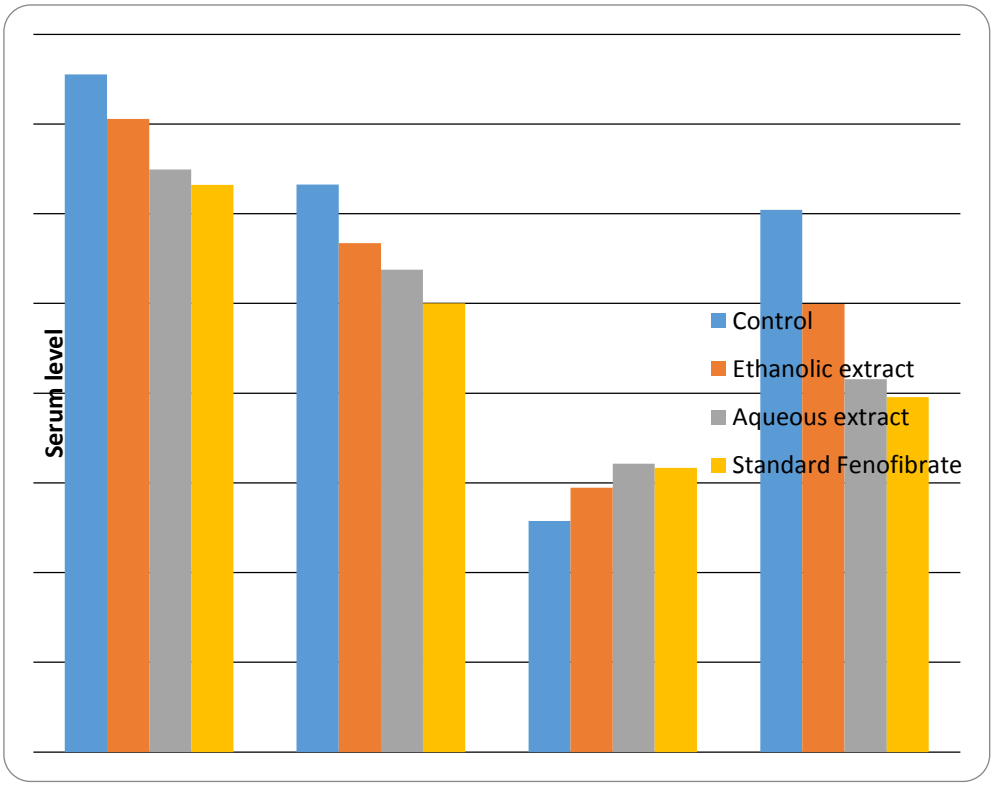

Fig No 15. Scheme of blood lipid profiles

7.4 Outcomes

The antihyperlipidaemic and antioxidant activity of the plant stem extract is studied and the significance is evaluated.

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples

Table No 12. Superoxide Radical Scavenging activity

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	73.50±0.70	46.46±0.34	24.23±1.45
50	77.63±3.12	56.47±0.86	38.01±0.34
75	82.33±0.96	65.00±0.87	48.44±0.14
100	85.93±0.79	70.23±1.46	59.36±1.11
150	93.30±0.02	73.56±1.76	68.36±1.08
200	94.54±0.06	75.22±0.60	70.57±0.59

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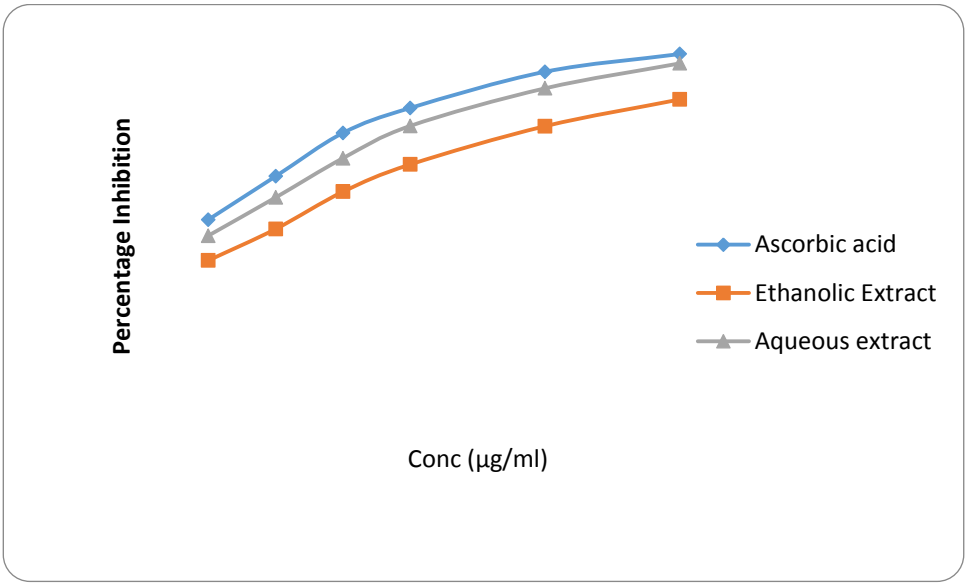

Fig No 16 . Schematic representation of superoxide radical scavenging activity of all the extracts

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples. **Table No 13. DPPH Assay**

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	66.20±0.22	61.25±0.29	66.24±0.90
50	70.44±0.57	62.54±0.62	68.52±0.13
75	76.22±0.67	64.22±0.56	72.44±2.76
100	78.44±0.29	67.77±0.56	77.34±1.56

150	87.47±1.05	74.24±2.33	85.59±1.99
200	92.02±0.02	76.23±0.24	88.33±1.97

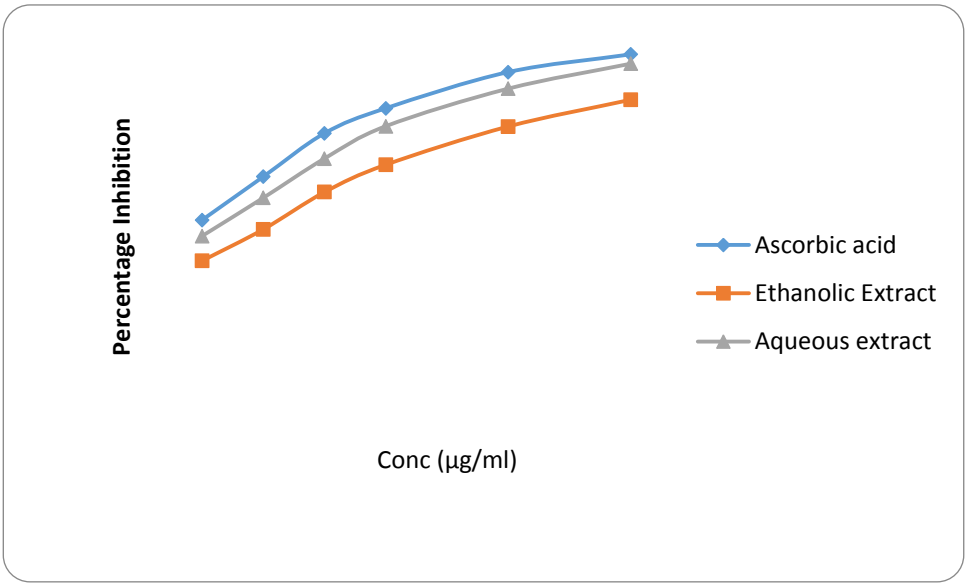

Fig No 17. Schematic representation of DPPH activity of all the extracts

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

Table No 14. Lipid per oxidation Assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	69.52±2.97	45.33±3.45	54.55±1.36
50	74.56±1.93	53.34±1.25	61.50±1.50

75	80.47±0.48	61.45±1.34	69.45±1.78
100	83.32±1.62	66.77±1.36	74.38±0.56
150	87.52±0.35	74.28±3.46	85.67±1.91
200	89.67±0.46	78.54±0.87	86.33±0.73

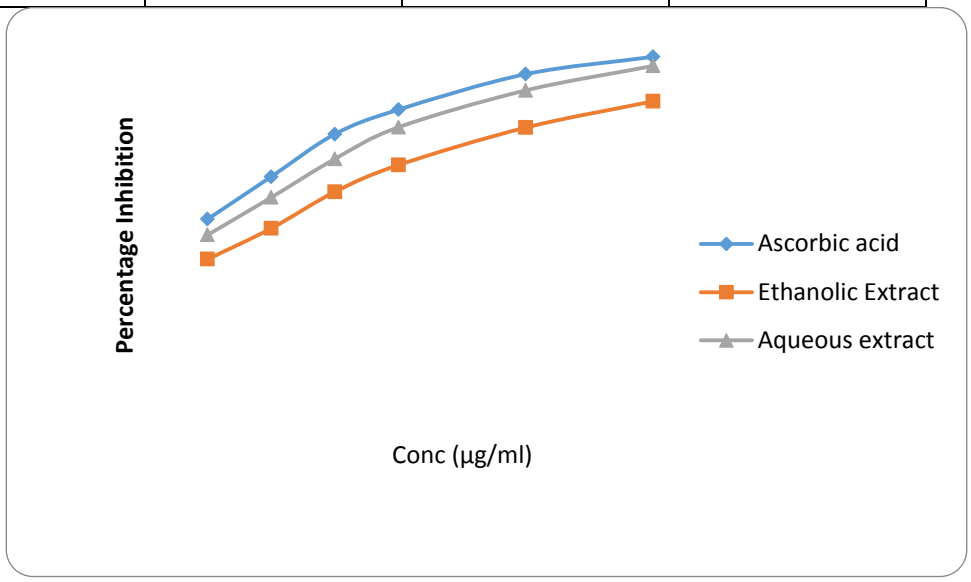

Fig No 18. Schematic representation of Lipid per oxidation Assay of all the extracts

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

Table No 15. Nitric oxide scavenging assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract
25	43.50±0.62	30.62±0.56	35.33±2.56

50	55.36±1.57	36.57±0.33	49.44±0.45
75	68.22±0.32	42.67±1.56	60.67±0.77
100	80.12±0.66	50.28±1.36	72.53±1.32
150	91.56±0.23	60.12±0.38	89.35±1.66
200	95.33±2.34	69.88±0.34	93.44±0.65

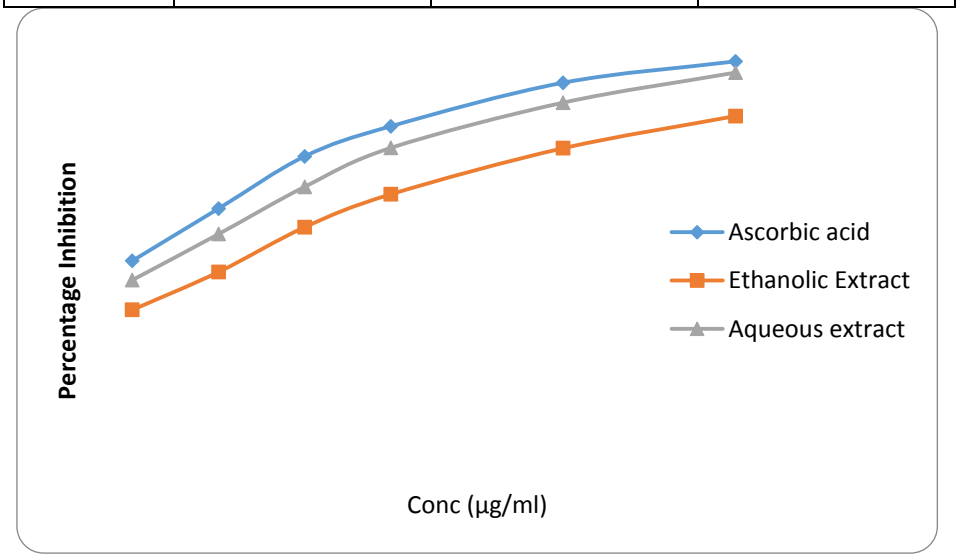

Fig No 19. Schematic representation of Nitric oxide scavenging assay of all the extra

**Ascorbic acid, ethanolic extract & aqueous extract in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the ethanolic and aqueous extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

Table No 16. Hydrogen per oxide assay

Conc. (µg/ml)	Ascorbic acid	Ethanolic Extract	Aqueous extract

25	48.66±0.22	39.56±0.34	45.09±0.42
50	58.32±0.68	46.58±1.26	53.60±1.52
75	67.99±1.66	54.88±1.62	62.36±1.67
100	73.56±0.12	60.97±1.42	69.56±0.79
150	81.62±0.26	69.49±1.31	77.93±1.74
200	85.57±0.12	75.45±0.38	83.49±0.57

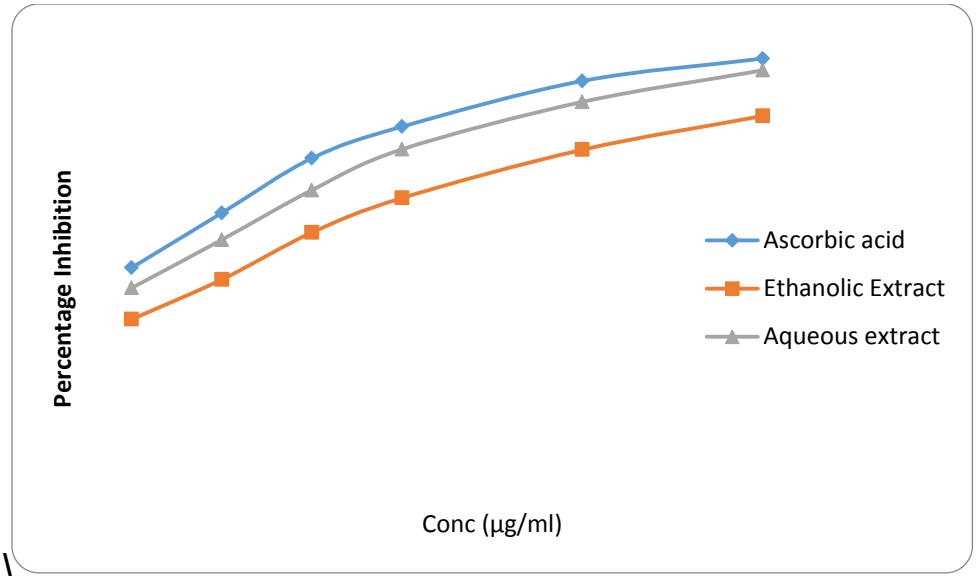

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Fig No 20. Schematic representation of Hydrogen peroxide assay of all the extracts

CHAPTER VIII

8. SUMMARY AND CONCLUSION

From the experimental and research revealing of the entire study of *Atrocarpus Hetrophyllus* indicated the presence of antihyperlipidemic activity and and the potential antioxidant activity as a whole. Various antioxidant invitro studies performed onto the *Atrocarpus Hetrophyllus* indicates that as compared to the ascorbic acid the radical scavenging activity are proven well, and it is comparatively equivalent to the Standard drug.

Animal studies performed on the particular extracts of *Atrocarpus Hetrophyllus* revealed that the antihyperlipidemic activity is very potential and found to be significantly reducing the triglycerides levels of all the animal group given with the prepared extracts, Hence the animal group administered with higher extract will be shown an increased activity as compared with the extract given in less equlavent and as compared with the standard antihyperglycemic drug as well.

Hence it is concluded that the potential benefits of the extracts of *Atrocarpus Hetrophyllus* has been demonstrated well in advance and can be used further to demonstrate the antihyperlipidemic as well as the antioxidant activity for the controlling of both triglyceride levels and reducing the risk of generation of ROS against the free radicals.

The aforementioned results of the research suggest that the *Atrocarpus Hetrophyllus,* found to have the potential antihyperlipidemic and antioxidant activity.

CHAPTER IX

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